FORMULATION AND EVALUATION OF DILTIAZEM HYDROCHLORIDE MICROSPHERES FOR ORAL CONTROLLED RELEASE DRUG DELIVERY USING POLY (ε-CAPROLACTONE)

Dissertation work submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the award of degree of

MASTER OF PHARMACY

(Pharmaceutics)

Submitted by
DIVIA.C

Under the guidance of
Mr.B. Rajalingam, M.Pharm.,(Ph.D)
Assistant Professor

March 2010

Department of Pharmaceutics
COLLEGE OF PHARMACY
SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES

COIMBATORE – 641044

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Certificate

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Coimbatore – 641 044.

Place: Coimbatore
Date :

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Chapter 7 Results & Discussion

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*Above all, I humbly submit my dissertation work, into the hands of **Almighty**, who is the source of all wisdom and knowledge for the successful completion of my thesis.*

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Divia.C

ABBREVIATIONS

SRDDS	Sustained Release Drud Delivery Systems
PCL	Poly (ε-caprolactone)
DTZ	Diltiazem
IP	Indian Pharmocopeia
IR	Infra Red spectrometer
Uv/vis	Ultra violet /visible
SEM	Scanning electron microscope
RT	Room temperature

CONTENTS

INTRODUCTION[1]

The concept of drug delivery has been revolutionized. The strides have been made to lend patient derive maximum benefits of a drug. The drug should be delivered to a specific target sites at a rate and concentration that permit optimal therapeutic efficacy while reducing side effects to minimum. Another aspect to be considered in drug delivery is patient compliance during the drug therapy.

The concept of the advanced drug delivery systems especially those offering a sustained and controlled action of drug to desired area of effect, attained great appeal for nearly half a century. However, prior to the advent of improved alternative methods, drug delivery systems were considered only as a means of getting the drug into patient's body. Actual practice of controlled release began with advent of timed release coating to the pills or solid drug particles in order to mask their unacceptable taste or make them palatable.

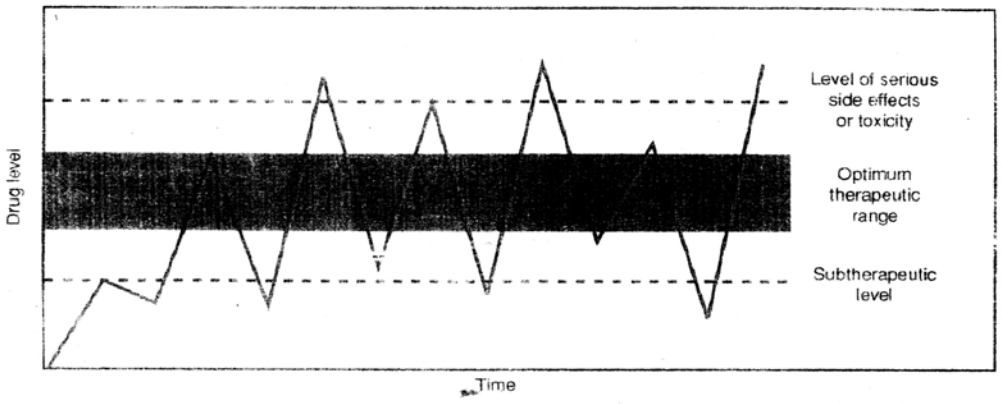
Chapter 7 Results & Discussion

Oral controlled release products are formulated to release active ingredient gradually and predictably over a 12 to 24hour period. These formulations potentially provide for greater effectiveness in the treatment of chronic conditions through more consistent delivery of the medication; reduced side effects; greater convenience; and higher levels of patient compliance due to a simplified dosage schedule, compared with those of immediate-release drugs.

DEFINITION[2]

Controlled drug delivery system is defined as the release of a drug or other active ingredient in a predesigned/ predetermined manner. The rationale for controlled delivery of drugs is to promote therapeutic benefits while at the same time minimizing toxic effects. Normal drug dosing follow a "**saw tooth**" kinetic profile, in which the dose first greatly exceeds the desired therapeutic level, then falls to subclinical level, and on subsequent dosing rises to dangerously high values, falling again to ineffective concentrations, in cycles of excessive- ineffective levels. Controlled, sustained drug delivery can reduce the undesirable fluctuation of drug levels, enhancing therapeutic action and eliminating dangerous side effects.

Fig. No.1: Undesirable sawtooth kinetic profile under conditions of normal dosing and optimum therapeutic profile obtainable with controlled release


Drug level
Level of serious side effects or toxicity
Optimum therapeutic range
Subtherapeutic level
Time

ADVANTAGES[2]

- More effective therapies.
- Elimination of the potential for both under and over dosing.
- Maintenance of drug levels within a desired range.
- The need for fewer administrations.
- Optimal use of drug in question.
- Increased patient compliance.

DISADVANTAGES[2]

- The possible toxicity or non biocompatibility of the materials used for the controlled release systems.
- Undesirable by-products of degradation.
- Any surgery required to implant or remove the system
- The chance of patient discomfort from the delivery device.
- The higher cost of controlled release systems compared with traditional pharmaceutical formulations.

IDEAL PROPERTIES[2]

Based on the mentioned advantages and disadvantages of controlled release, one might formulate requirements for the "ideal" drug delivery system as follows. Such systems should be

- Inert
- Biocompatible
- Mechanically strong
- Comfortable for the patient
- Capable of achieving high drug loading
- Safe from accidental drug release
- Simple to administer and remove
- Easy to fabricate and sterilize

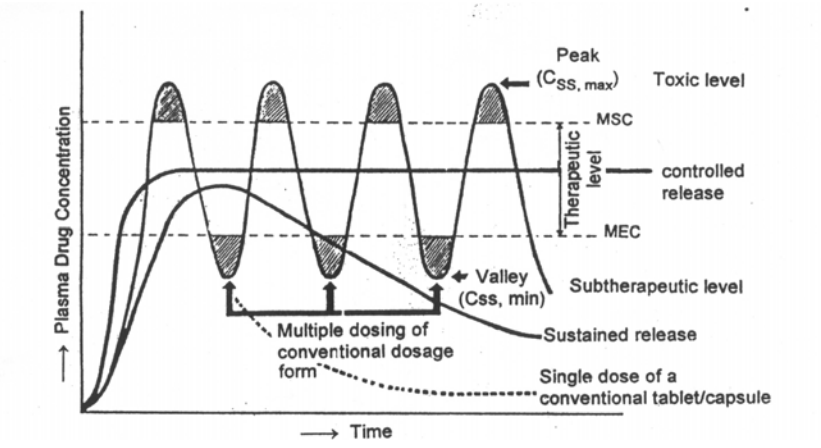
If one were to imagine the ideal drug delivery system, two prerequisites would be required. First, it would be a single dose for duration of treatment, whether it is for days or week, as in infections, or for lifetime of the patient, as in hypertension or diabetes. Second, it should deliver the active entity (drug) directly to the site of action, thereby minimizing or eliminating side effects. This may necessitate delivery to specific receptors or to localization to cells or to specific areas of the body[3].

The goal of many of the original sustained controlled release systems was to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. The key point with traditional drug administration is that the blood level of the agent should remain between a maximum value which may represent a toxic level, and a minimum value below which the drug is no longer effective. In controlled drug delivery systems designed

for long term administration and sustained drug release, the drug level in the blood remains relatively constant, between a desired maximum and minimum, for an extended period of time.

It is obvious that this imaginary delivery system will have changing requirements for different disease states and different drugs. Thus, we wish to deliver the therapeutic agent to a specific site and for a specific time. In other words, the objective is to achieve both **spatial** and **temporal** placement of drug. Currently, it is possible to achieve both of these goals, with most drug delivery systems. The given pictorial representation in **Fig No: 2** gives an idea about the sustained drug delivery system[4].

Fig.No. 2: plasma concentration Vs time profile



FACTORS GOVERNING THE DESIGN OF SRDDS[1]

There are number of factors which may influence the design of any dosage form. Similarly design of sustained release dosage

form is governed by the factors listed below in **table No: 1**

Table.No.1: Factors governing the design of SRDDS

Drug related Physicochemical properties of drug	Aqueous solubility Partition coefficient Protein binding Molecular weight Drug stability
Pharmacokinetic	Absorption rate Elimination half life Rate of metabolism Dosage form index(DI) First pass metabolism
Pharmacodynamic	Therapeutic range Therapeutic index(TI) Plasma-concentration responses
Route of administration	Dose size Absorption efficiency Duration of action
Pharmacological	Changes in drug effect upon multiple dosing Sensitizing Tolerance
Physiological	Prolonged drug absorption Variability in GI emptying and motility GI blood flow

CRITERIA OF DRUG SELECTION FOR SRDDS[5,6]

Characteristics of Drugs Unsuitable for oral SRDDS

- Not effectively absorbed in the lower intestine

  (eg: Riboflavin, Ferrous salts, etc)

- Absorbed and excreted rapidly; short biologic half-lives

  (< 1hr) (eg: Penicillin G, furosemide, etc)

- Long biologic half-lives (>12 hr) (eg:Diazepam, phenytoin, etc)
- Large doses required (>1g) (eg: Sulfonamides)
- Cumulative action and undesirable side effects; drugs with low therapeutic indices (eg: Phenobarbital, digitoxin, etc)
- Precise dosage titrated to individual is required (eg: Anticoagulants, cardiac glycosides, etc)
- No clear advantage for sustained release formulation (eg: Griseofulvin)

The following are the criteria to be met by drug proposed to be formulated in sustained release dosage forms.

a) Desirable half-life

The half life of a drug is an index of its residence time in the body. If the drug has a short half life (less than 2 hours), the dosage form may contain a prohibitively large quantity of the drug. On the other hand, drug with elimination half life of eight hours or more are sufficiently sustained in the body, when administered in conventional dosage from, and controlled release drug delivery system is generally not necessary in such cases. Ideally, the drug should have half-life of three to four hours.

b) High Therapeutic Index

Drugs with low therapeutic index (eg: digitoxin) are unsuitable for incorporation in controlled release formulations. If the system fails in the body, dose dumping may occur, leading to

fatalities (eg. Digitoxin).

c) Small dose

If the dose of a drug in the conventional dosage form is high(>1g), its suitability as a candidate for controlled release is seriously undetermined (eg: antibiotics). This is chiefly because the size of a unit dose of new delivery system would become too big, to administer with difficulty.

d) Desirable absorption and solubility characteristics

Absorption of poorly water soluble drug is often dissolution rate limited. Incorporating such compounds into controlled release formulations is therefore unrealistic and may reduce overall absorption efficiency.

e) First pass clearance

Delivery of the drug to the body in desired concentrations is seriously hampered in case of drugs undergoing extensive hepatic first pass metabolism, when administered in controlled release forms.

MICROSPHERES/ MICROCAPSULES[1]

The term microcapsule is defined as a spherical particle with size varying from 50nm to 2mm containing a core substance. Microspheres are in strict sense, spherical empty particles. However the term microcapsules and microspheres are often used synonymously. The microspheres are characteristically free flowing

powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200nm. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for the controlled release of drug.

DEVELOPMENT OF A MICROENCAPSULATION PROCEDURE[7]

The microspheres can be prepared by using any of several techniques discussed in the following sections but the choice of the technique mainly depends on the nature of the polymer used the drug, the intended use and the duration of therapy. Moreover, the method of preparation and its choice are equivocally determined by some formulation and technology related factors as mentioned below:

- The particle size the final product required.
- The drug or the protein should not be adversely affected by the process.
- Reproducibility of the release profile and the method.
- No stability problem.
- There should be no toxic product associated with the final product.

Different types of methods are employed for the preparation of the microspheres. These include single emulsion technique, double emulsion technique, *in-situ* polymerization, solvent

evaporation, coacervation phase separation, spray drying, spray congealing etc.

SINGLE EMULSION TECHNIQUE[1]

The microparticulate carriers of natural polymers, i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in the non-aqueous medium. In the second step of preparation, cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using chemical cross linkers like glutaraldehyde, formaldehyde, diacid chloride etc. cross-linking by host is affected by adding the dispersion to previously heated oil. Heat denaturation is however, not suitable for the thermolabile drugs while the chemical cross-linking suffers disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation.

DOUBLE EMULSION TECHNIQUE[1]

Double emulsion method of microspheres preparation involves the formation of multiple emulsions or the double emulsion of the type w/o/w and is best suited to water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as synthetic polymers. The

aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is then subjected to homogenization or sonication before addition to the aqueous solution of poly vinyl alcohol. This results in the formation of a double emulsion and the resultant is then subjected to solvent removal either by solvent evaporation or solvent extraction.

POLYMERIZATION TECHNIQUES[1]

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

- **Normal polymerization**

Normal polymerization proceeds and carried out using different techniques as bulk, suspension, emulsion and miceller polymerization processes.

- **Interfacial polymerization**

Interfacial polymerization essentially proceeds involving reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase.

- **Phase separation coacervation technique**

The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of the polymer rich phase called coacervates. The coacervation can be brought about by addition of the third component to the system which results in the formation of the two phases, one rich in the polymer, while the other one, i.e. supernatant, deplete of the polymer. There are various means and methods, which are effectively employed for coacervate phase separation like salt addition, non-solvent addition, addition of incompatible polymer, change in pH etc. The method choice is largely dependent upon the polymer and set of conditions.

- **Spray drying and spray congealing**

Spray drying and spray congealing methods are based on the drying of the mist of the polymer and drug in the air. Depending on the removal of the solvent or the cooling of the solution, the two processes are named spray drying and spray congealing respectively.

- **Solvent evaporation technique[7]**

The oil-in-water (o/w) solvent evaporation method, also known as "**in-water drying**", originally developed for the encapsulation of water insoluble drugs. The method involves the preparation of a solution of a wall forming polymer in a water-

immiscible organic solvent into which the drug is dissolved directly or with the aid of a cosolvent or dispersed in a fine state. This is then added in a controlled fashion into an aqueous solution of an emulsifying agent under intense agitation. It is generally not applicable to the encapsulation of highly water soluble peptides within hydrophobic polymers because upon emulsification of the dispersion of the drug-organic polymer solution/dispersion into the external aqueous phase, most of the peptides partitions out into the external phase resulting into negligible entrapment in the microspheres.

In 1970 a multiple emulsion solvent evaporation microencapsulation procedure was patented by Vrancken and Claeys and further by DeJaeger and Tavernier in 1971. In brief, an aqueous solution of the drug substance was emulsified under high-speed homogenization or sonication into a solution of polymer in an organic solvent. This emulsion, known as the primary emulsion, was then poured under constant stirring into an external aqueous praise containing a suitable emulsifier.

For successful development of a microencapsulation procedure it is essential to have an excellent understanding and control on the polymer and its chemistry.

POLYMERS[7]

One of the preliminary requirements in the successful

development of a microencapsulation procedure and in achieving a product of reproducible quality in terms of microencapsulation efficiency, yield, scale-up performance, and finally, drug release characteristics is the selection of a suitable polymer as the coating material and the complete characterization of the polymer.

The requirements for biodegradable polymer for drug delivery include controlled biodegradation rate, production of nontoxic degradation products and metabolites, reproducible and economically viable manufacturing process for large scale manufacture, absence of impurities such as residual solvents, catalysts, monomers, stabilizers etc and ease of processing

PREREQUISITES FOR IDEAL MICROPARTICULATE CARRIERS[1]

The material utilized for the preparation of microparticulates should ideally fulfill the following prerequisites.

- Longer duration of action
- Control of content release
- Protection of drug
- Reduction of toxicity
- Biocompatibility
- Sterilizability
- Relative stability
- Bioresorbability
- Targetability
- Tensile strength
- Poly valent
- Transition temperature
- Increase of therapeutic efficiency
- Water solubility or dispersability

CLASSIFICATION OF POLYMERS

The polymers are the important component as it decides the release of drug from sustained release dosage forms. The polymers are basically classified as

1. Biodegradable
2. Non biodegradable

Table No.2: Classification of polymers

Biodegradable polymers	Non biodegradable polymers
Poly esters eg: poly(glycolic acid), poly(lactic acid), poly(caprolactone), etc	Poly ethylene vinyl acetate (EVA)
Natural polymers eg: collagen, gelatin, albumin, starch, chitosan ,etc	Poly ether urethane (PEU)
Synthetic eg: poly amino acids, poly alkyl cyano acrylate, poly amides etc	Poly vinyl chloride (PVC)

Biodegradable polymers for microparticles

The biodegradable polymers comprised of monomers linked to one another through functional groups and have unstable linkages in the backbone. They are biologically degraded or eroded by enzymes or generated by surrounding living cells.

Biodegradable microparticles allow the drug release to be accurately tuned for the treatment of the Specific disease through the appropriate choice and formulation of specific drugs and polymers. Based on various microencapsulation techniques,

microparticles can be designed for optimum delivery of a selected bioactive agent. The resulting microparticles may offer the ability to improve the stability of therapeutic agents against hydrolytic or enzymatic degradation, to augment the therapeutic effect by releasing the drug into the specific site, and to sustain the therapeutic effect in the target site. Many synthetic and natural biodegradable polymers present exciting opportunities in tailor-making the microparticle formulations for long-term drug release with specific release rates.

THE ORGANIC SOLVENT[7]

In addition to the choice of the proper polymer for microencapsulation it is also essential to determine the appropriate solvent for the preparation. The selection of the solvent and the external continuous phase determine microsphere formation and entrapment efficiencies. A good solvent for microencapsulation should have the following properties:

1) Good solvency of the polymer
2) Poor solvency of the drug
3) Low boiling point
4) Should not cause the degradation of the drug substance
5) Should be acceptable for human use

THE EXTERNAL PHASE[7]

The external phase in a solvent evaporation encapsulation method should be inexpensive, high boiling, non toxic and immiscible with organic solvent. The external phase should also contain an emulsifier. As the solvent evaporation proceeds to a completion the droplets generated initially shrink in size as the organic solvent evaporates. During this early evaporation stage the droplets tend to coalesce and form agglomerates. A good emulsifier is required for the stabilization of the droplets to prevent coalescence by the formation of a thin film. As the evaporation proceeds, the emulsifier film helps to maintain the spherical shape of the droplets till such time as the droplets are hardened enough to be harvested.

DRUG RELEASE KINETICS[1]

Release of the active constituent is an important consideration in case of microspheres. Many theoretically possible mechanisms may be considered for the release of drug from microparticulates.

- Liberation due to polymer erosion or degradation.
- Self diffusion through the pore.
- Release from the surface of the polymer.
- Pulsed delivery initiated by the application of an oscillating or sonic field.

Chapter 7 Results & Discussion

In most of the cases, a combination of more than one mechanism for drug of release may operate so the distinction amongst the mechanisms is not always trivial. The release profile from the microspheres depends on the nature of the polymer used in the preparation as well as nature of the active drug. The release of drug from both biodegradable as well as non biodegradable microspheres is influenced by structure or micro- morphology of the carrier and the properties of the polymer itself.

Factors affecting the release of the drug from the particulate system in relation to drug, microspheres and bioenvironment:

- Drug
- Position in microspheres
- Molecular weight
- Physicochemical properties
- Concentration
- Interaction with matrix
- Microspheres
- Type and amount of polymer
- Size and density of the microspheres
- Extent of cross linking, denaturation or polymerization
- Environment

- pH
- Polarity
- Presence of enzyme

RESERVOIR TYPE SYSTEM

Release from the reservoir type system with rate controlling membrane proceeds by first penetration of the water through the membrane followed by dissolution of the drug in the penetrating dissolution fluid. The dissolved drug after partitioning through the membrane diffuses across the stagnant diffusion layer. The release is essentially governed by the Fick's first law of diffusion as

$J=-D\ (dc/dx)$

Where, J is flux per unit area

D = diffusion coefficient

(dc/dx) = concentration gradient

Diffusion across the membrane determines the effectiveness of the carrier system. The cumulative amount of drug that is released through the unit area, 'Q_t' at any time 't' is given by equation:

$$Q_t = C_s K D_m D_d t / K D_m I_m + D_d I_d$$

Where, C_s = saturation solubility of drug in dispersion medium

D_m = diffusion coefficient of drug in membrane of thickness

I_m

D_d = diffusion coefficient of drug in static diffusion layer of thickness I_d

K = partition coefficient of drug between membrane and reservoir compartments.

The release rate from the carriers can be modified by changing both the composition and the thickness of the polymeric membrane.

MATRIX TYPE SYSTEM

Release profile of the drug from the matrix type of the device critically depends on the state of drug whether it is dissolved or dispersed in the polymer matrix. In case of the drug dissolved in the polymeric matrix, amount of drug, and the nature of the polymer (whether hydrophobic or hydrophilic) affect the release profile.

In case of drug dissolved in the polymeric matrix, the amount of drug appearing in the receptor phase at time't' is approximated by two separate equations. The first equation determines the initial 60 percent of the drug release while the second shows the release profile at the later stage.

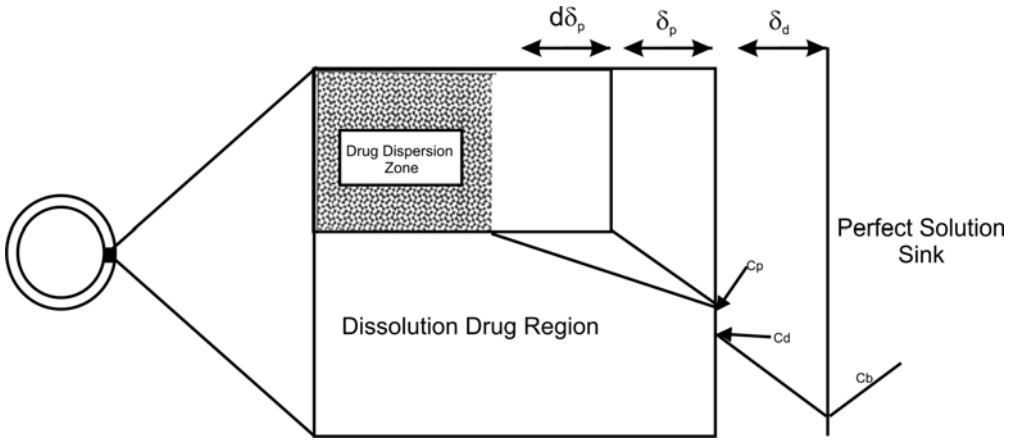


Fig.No.3 : Schematic Representation of Controlled Drug Molecules from a Matrix type drug delivery devices

$dMt/dt = 2M_x(D/\pi l^2 t)^{t/2}$

$dM_t/dt = 8DM_x/l^2 exp\pi^2 Dt/l^2$

Where, l = thickness of polymer slab

D = diffusion coefficient

M_x = total amount of the drug present in the matrix

M_t = amount of the drug released in time t

When the drug is dispersed throughout the polymer matrix then the release profile follows Higuchi's equation:

$dMt/dt = A/2(2DC_sC_o)^{1/2}/t$

Where, A = area of matrix

C_s = solubility of the drug in the matrix

C_o = total concentration in the matrix.

Taking porosity (ε) and tortuosity (r) of the matrix into the

consideration the above equation can be rewritten as $dM_t/dt = [\varepsilon/r\ D_m(2C_o-\varepsilon C_s)C_s t]^{1/2}$

POLYMER PROFILE

POLY (ε-CAPROLACTONE)[35,36]

General introduction

Poly (ε- caprolactone) (PCL) is a semicrystalline polyester biodegradable polymer which comes under the category of aliphatic polyester. Aliphatic polyesters are a group of synthesized, nontoxic, biodegradable polymers. They are synthetic homopolymers or copolymers of lactic acid, glycolic acid and ε-hydroxycaproic acid. Polycaprolactone (PCL) is of great interest as it can be obtained by the ROP of a relatively cheap monomeric unit 'e-caprolactone'. The PCL is highly processible as it is soluble in a wide range of organic solvents while having the ability to form miscible blends with wide range of polymers. Typically, the molecular weights of homopolymers and co-polymers range from 2000 to >100000.

The rate of biodegradation and drug-release characteristics from these systems formulated with the aliphatic polyesters can be controlled by changing the physicochemical properties of the polymers, such as crystallinity, hydrophobicity, monomer stereochemistry, co-polymer ratio, and polymer molecular weight.

Chapter 7 Results & Discussion

Synonym

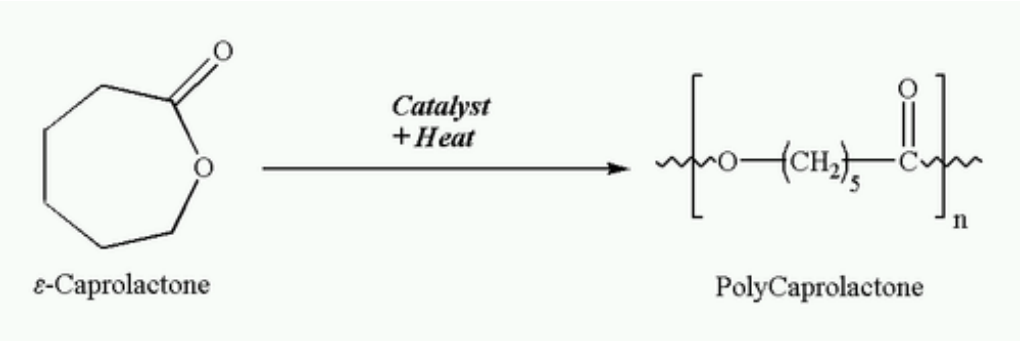
PCL

Chemical name

2- oxypanone

Chemistry

Structure :



Description

Molecular weight	:	80 – 150000
Melting point	:	58 – 63˚C
Glass transition temperature	:	-65 to -60˚C
Colour	:	White
Solubility	:	dichloromethane, chloroform,
Specific gravity	:	1.11
Tensile strength	:	3000 – 5000psi
Elongation (%)	:	300 – 500%

Modulus (Psi) : $3 – 5 \times 10^7$psi

FUNCTIONAL CATEGORY

Bioabsorbable, biocompatible, biodegradable material

STABILITY AND STORAGE CONDITIONS

The aliphatic polyesters are easily susceptible to hydrolysis in the presence of moisture. Hence they should be properly stored, preferably refrigerated at below 0˚C. It is necessary to allow the polymers to each room temperature before opening the containers. After the original package has been opened, it is recommended to re-purge the package with high purity dry nitrogen prior to resealing.

APPLICATIONS IN PHARMACEUTICAL FORMULATION OR TECHNOLOGY

Aliphatic polyesters are a group of synthesized, non toxic, biodegradable polymers. In an aqueous environment, they undergo hydrolytic degradation, through cleavage of the ester linkages, into non toxic hydroxyl carboxylic acids. Aliphatic polyesters are eventually metabolized to carbondioxide and water, via the citric acid cycle. As the polymer undergoes hydrolytic degradation due to the presence of hydrolytically labile aliphatic ester linkages; however, the rate of degradation is rather slow (2–3 years).

Due to the slow degradation, high permeability to many drugs and non-toxicity, PCL was initially investigated as a long-term drug/vaccine delivery vehicle. Owing to their reputation as safe materials and their biodegradability, aliphatic polyesters are primarily used as biocompatible and biodegradable polymers for formulation of many types of implantable and injectable drug delivery systems for both human and vetenary use. Examples of implantable drug delivery systems include rods, cylinders, tubing, films, fibres, pellets and beads. Examples of injectable drug delivery system include microcapsules, microspheres, nanoparticles and liquid injectable controlled release systems. The rate of biodegradation and drug release characteristics from these systems formulated with the aliphatic polyesters can be controlled by changing the physicochemical properties of the polymers such as crystallinity, hydrophobicity, monomer stereochemistry, copolymer ratio and polymer molecular weight. The long-term contraceptive device Capronors is composed of this polymer and has been developed for the long-term zero order release of levonorgestrel. PCL has low tensile strength (approximately 23MPa) but an extremely high elongation at

breakage (4700%).Extensive research is ongoing to develop various micro- and nano-sized drug delivery vehicles based on PCL. Due to its excellent biocompatibility, PCL has also been extensively investigated as scaffolds for tissue engineering. A recent study demonstrated the feasibility of using a composite matrix composed of PCL and hyaluronic acid as a potential meniscus substitute. Composites of PCL with calcium phosphate based ceramics are also currently being investigated as suitable scaffolds for bone tissue engineering.

Safety

Poly(ε-caprolactone) is used in parentral pharmaceutical formulations and are regarded as biodegradable, biocompatible and bioabsorbable materials. Their biodegradation products are non toxic, non carcinogenic and non teratogenic. In general, these polyesters exhibit very little hazard.

Handling precautions

Observe normal precautions appropriate to circumstances and quantity of material handled. Contact with eyes, skin and clothing, and breathing the dust of the polymers should be avoided. Aliphatic polyesters produce acid materials such as hydroxyacetic and/ or lactic acid in presence of moisture; thus,

contact with materials that will react with acids, especially in moist condition should be avoided.

DRUG PROFILE

DILTIAZEM HYDROCHLORIDE[32-34]

Diltiazem hydrochloride is a member of the group of drugs known as benzothiazepines, which are a class of calcium channel blockers.

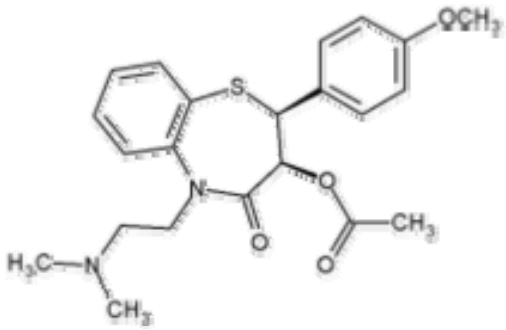
Chemical name

Cis-(+)-[2-(2-dimethylaminoethyl)-5-(4-methoxyphenyl)-3-oxo-6-thia-2-azabicyclo[5.4.0]undeca-7,9,11-trien-4-yl]ethanoate

Empirical formula

$C_{22}H_{26}N_2O_4S.Hcl$

Chemical structure



.Hcl

Description

Colour : White

Solubility	:	Freely soluble in water, methanol, chloroform, slightly soluble in ethanol, insoluble in benzene
Melting point	:	207.5-212˚C
Molecular weight	:	450.98
Half life	:	3-4.5 hrs

Dosing information

- **Adults**

In atrial arrhythmia an IV bolus, initial 0.25 mg/kg (or 20 mg) IV over 2 min and IV continuous infusion, initial 5-10 mg/hr; increase in 5 mg/hr increments up to 15 mg/hr maintained for up to 24 hr.

In hypertension, Sustained release initial 60-120 mg orally twice daily; usual dose 120-180 mg twice daily, maximum 360 mg/day. Extended release initial 120-240 mg orally once daily: titrate after 14 days: usual dose, 240-360 mg orally once daily, maximum 540 mg/day

- **Pediatric**

Not FDA- approved in pediatric patients.

MECHANISM

- Diltiazem is a potent vasodilator, increasing blood flow and variably decreasing the heart rate via strong depression of A-V node conduction. Its pharmacological activity is somewhat similar to verapamil.
- Potent vasodilator of coronary vessels.
- Vasodilator of peripheral vessels. This reduces peripheral resistance and afterload.
- Negative inotropic effect. Diltiazem causes a modest decrease in heart muscle contractility and reduces myocardium oxygen consumption.
- Negative chronotropic effect. Diltiazem causes a modest lowering of heart rate. This effect is due to slowing of the SA (sinoatrial) node. It results in reduced myocardium oxygen consumption.
- Negative dromotropic effect. By slowing conduction through the AV (atrioventricular) node, diltiazem increases the time needed for each beat. This results in reduced myocardium oxygen consumption by the body.

Nontherapeutic effects and toxicities

Reflex sympathetic response. Caused by the peripheral dilation of vessels and the resulting drop in BP; the response works to counteract the inotropic, chronotropic and dromotropic effects of diltiazem. Symptoms include hypotension, bradycardia, dizziness, flushing.

Contraindications and precautions

- Congestive heart failure. Patients with reduced ventricular function may not be able to counteract the inotropic and chronotropic effects of diltiazem, the result being an even higher compromise of function.
- SA node or AV conduction disturbances. Use of diltiazem should be avoided in patients with SA or AV nodal abnormalities, because of its negative chronotropic and dromotropic effects Low blood pressure. Patients with systolic blood pressures below 90 mm Hg should not be treated with diltiazem.
- Wolff-Parkinson-White syndrome. Diltiazem may paradoxically increase ventricular rate in patients with WPW syndrome because of accessory conduction pathways.
- Diltiazem is relatively contraindicated in the presence of sick sinus syndrome, atrioventricular node conduction disturbances, bradycardia, impaired left ventricle function, peripheral artery occlusive disease, chronic obstructive pulmonary disease, and Prinzmetal's angina.

PHARMACOKINETIC PROPERTIES

Absorption

90% administered dose is absorbed but extensive first pass metabolism limits the absolute bioavailability to 30-40%. Relative to an intravenous dose large patient-to-patient variation in the plasma levels achieved with a single oral dose, consistent with a large first-pass metabolism or individual differences in absorption.

Distribution

- **Distribution sites**

- Protein binding : 77% to 93% where diltiazem hydrochloride binds with albumin in the range of 35 to 40%.Protein binding is independent of serum diltiazem hydrochloride concentrations and therapeutic serum levels of digoxin, hydrochlorthiazide, phenylbutazone, propranolol, salicylic acid and warfarin do not influence the percentage of unbound diltiazem.

- **Distribution kinetics**

- Distribution half-life : 0.3 hours
- Volume of distribution: 5.3L/kg (300 to 400 litres)

Metabolism

DTZ primarily gets metabolized in liver through deacetylation. The metabolites of DTZ are Deacetyl diltiazem (active) which is the major metabolite; present in the plasma at levels of 10% to 45% of the parent; 25% to 50% as a potent coronary vasodilator and N-monodesmethyldiltiazem (inactive) which accumulates more than desacetyldiltiazem at steady state.

Excretion

- 35% of DTZ undergoes Renal excretion. Only 1% to 3% as unchanged diltiazem, the bulk as metabolites. Total body clearance : 11.8 mL/minute/kg(2 fold decrease after repeated dose). Value may be up to 2-fold lower after repeated dosing.

Elimination half-life

For parent compound elimination half- life was 3.06 to 6.6 hours and for extended release formulation 4 to 10 hours. All extended or controlled release dosage forms report similar ranges (4 to 9.5; 5 to 7;5 to 10 hours) of apparent half-life following both single and multiple doses. Half-life of diltiazem long acting tablets is 6 to 9 hours.

PRECAUTIONS

- Coadministration with other drugs known to decrease peripheral resistance, intravascular volume or myocardial contractility or conduction.
- Concomitant use of beta blockers or digitalis; additive effect on heart rate.
- Dermatologic reactions leading to erythema multiforme and/or exfoliative dermatitis.
- Hepatic impairment; increased risk of toxicity.
- Hypotension
- Renal impairment; increased risk of toxicity.
- Supraventricular arrhythmias with hemodynamic compromise.
- Ventricular function impaired; worsening congestive heart failure.

ADVERSE EFFECTS

- **Mild adverse effects**

  Allergic reactions : Skin rash, hives, itching

  Other reaction : Headache, drowsiness, dizziness, nervousness, depression, confusion, hallucination

- **Severe adverse effects**

Asystole, bradyarrhythmia. Cardiac disrhythmia, congestive heart failure, edema, heart block, vasculitis, hypotension, myocardial infarction.

REVIEW OF LITERATURE

S.Jayaprakash *et al.*,[8] (2009), in their work **"Preparation and evaluation of biodegradable microspheres of Methotrexate"** reported that sustained release methotrexate microspheres of bovine serum albumin were prepared in different ratios by emulsion cross linking method. The prepared microspheres were subjected to various physicochemical evaluation and *in-vitro* release studies. The drug release from microspheres of 1:6 ratio was found with most constant and prolonged drug release and it follows diffusion by erosion mechanism. The characteristics of prepared microspheres are conductive to the formulation of the sustained release drug delivery system.

Yodthong Baimark *et al.*,[9] (2009), in their work

"Preparation of organic solvent/ surfactant free microspheres of methoxy poly(ethylene glycol)-b-poly(ε-caprolactone) by a melt dispersion method" reported that the microspheres were produced in 90-100°C glycerol by melt dispersion method. Morphology of the microspheres was spherical in shape with rough surfaces. Almost microspheres were in the size range of 300-500 μm. Microsphere cross-sections showed condensed phases throughout the microsphere matrices. Melting temperatures and heats of melting of the MPEG-*b*-PCL were decreased in the microsphere form. In conclusion, the use of melt dispersion method results in organic solvent and surfactant-free biodegradable microspheres of diblock copolymer that showing a potentially useful drug delivery systems with free from surfactants and organic solvents.

Nazar Mohammad Ranjha *et al.*,[10] (2009), in their work **"Encapsulation and characterization of Flubiprofen loaded poly(ε-caprolactone)-poly(vinylpyrollidone) blend microspheres by solvent evaporation method"** reported that Flurbiprofen loaded PCL/PVP blend microspheres were prepared by o/w solvent evaporation method. Microsphere recovery decreased with a decrease in the concentration of the emulsifier in the dispersion. Encapsulation efficiency and drug loading of microspheres increased with decrease in concentration of emulsifying agent.

Hydration rate, encapsulation efficiency and drug loading of microspheres increased with increase in concentration of PVP. SEM photographs revealed microspheres were discrete, spherical and became porous with decrease in concentration of emulsifying agent and vice versa. FTIR spectra of pure and encapsulated flurbiprofen in all formulation showed no significant difference in characteristic peaks, suggesting stability of flurbiprofen during encapsulation process. X-RD (X-ray powder diffractometry) of pure flurbiprofen shows sharp peaks, which decreases on encapsulation, indicating dispersion at molecular level and hence decrease in the crystallinity of drug in microspheres. Microspheres had shown an enteric nature at pH 1.2 and a sustained release pattern at pH 6.8. Rapid drug release was observed in microspheres with higher concentration of PVP (polyvinylpyrrolidone). Drug release kinetics followed zero order at pH 1.2 while at pH 6.8 Higuchi model was best fitted and was found non fickian.

Jeevana J.B *et al.*,[11](2009), in their **work " Development and Evaluation of Gelatin microspheres of Tramadol Hydrochloride"** reported that Tramadol hydrochloride could be encapsulated into gelatin microspheres with an entrapment efficiency of 97.2%. Spherical, transparent and free flowing microspheres were obtained. SEM revealed the spherical

structures. The FTIR and DSC analysis indicated the stability and compatibility of the drug in gelatin microspheres. The microspheres were in the suitable particle size range of 20-160µm. the drug was released continuously for a period of 12 hrs with a maximum release of 99.79%.

S.Thamizharasi *et al.*,[12] (2008), in their work "**Formulation and evaluation of Pentoxyfylline loaded poly (e-caprolactone) microspheres**" reported that pentoxyfylline loaded poly(ε-caprolactone) microspheres were prepared by solvent evaporation technique with different drug to carrier ratio [(1:3), (1:4), (1:5) and (1:6)]. The shape of microspheres were found to be spherical [SEM]. The size of microspheres were found to be ranging 59.3±6.3µm to 86.22±4.23µm. Among the four drug to carrier ratio 1:6 showed maximum percentage yield and 1:4 showed highest drug entrapment. The release followed Higuchi kinetics indicating diffusion controlled drug release.

S. Ravi *et al.*,[13] (2008), in their *work* **"Development and characterization of polymeric microspheres for controlled release protein loaded drug delivery system"** reported that the hydrophilic bovine serum albumin was chosen as a model protein to be encapsulated with poly (D,L-lactide-co-glycolide) (50:50) microspheres using a w/o/w double emulsion solvent evaporation

method. The microspheres prepared with different molecular weight and hydrophilicity of poly (D,L-lactide-co-glycolide) were found to be non porous smooth surfaced and spherical in structure under SEM with a mean particle size ranging from 3.98 to 8.74μm. The protein loading efficiency varied from 40 to 71% of the theoretical amount incorporated. The *in vitro* release profile of bovine serum albumin from microspheres presented two phases, initial burst release phase due to the protein absorbed on microsphere surface, followed by slower and continuous release phase corresponding to the protein entrapped in polymer matrix.

Parasuram Rajam Radhika *et al.*,[14] (2008), in their work **"Preparation and evaluation of delayed release Aceclofenac microspheres"** reported that delayed release microspheres of aceclofenac were formulated using an enteric polymer, cellulose acetate phthalate (CAP) prepared by solvent evaporation technique. The effects of various other modern enteric polymers such as hydroxyl propyl methyl phthalate cellulose (HPMCP), eudragit L 100 and eudragit S100 on the release of aceclofenac from the CAP microspheres have been evaluated. The microspheres were characterized for particle size, scanning electron microscopy(SEM), percentage yield, drug entrapment and *in vitro* release kinetics. The shape of microspheres were found to be spherical. The drug entrapment efficiency of microspheres was found to be ranging

from 75.65 to 96.52%w/w. The results also revealed that HPMCP exhibits positive influence where as eudragit L 100 and eudragit S 100 exhibits negative effect on the drug release rate of CAP microspheres. *In-vitro* drug release from all formulations followed the first order release kinetics and erosion plot.

A.V.Yadav *et al.*,[15] (2008), in their work **"Development of biodegradable starch microspheres for intra nasal delivery"** reported that spherical microspheres were obtained in all batches with mean diameter in the range of above 22.8 to 102.63µm. They showed a good mucoadhesive property and swelling behavior. The *in-vitro* release was fond in the range of 73.11-86.21%w/w. Concentration of both polymer and drug affect *in-vitro* release of drug from the microspheres.

Rima Kassab *et al.*,[16] (2008), in their work "**Formulation of Modified Microspheres Based on Cyclodextrin-Lactic Acid Polymers"** reported that Polymers, based on Poly L-lactic acid (L-PLA) and coupled with β-Cyclodextrin (β-CD), have been used for the preparation of microspheres for drug encapsulation. The strategy was based on the modification of the terminal carboxylic group of L-PLA (73.000) by coupling it with a β-CD in the presence of the peptide coupling agents: DCC/HOBT. The degree of functionalisation was found to be 80%. Characterizations of the new product were carried out using 1H NMR, gel permeation

chromatography, and acid base titration. The size of the functionalized microspheres were determined to be 211 μm by Dynamic Light Scattering (DLS). Amphotericin B (AmB), a polyenic antifungal molecule, has been incorporated in L-PLA coupled with β-CD microspheres. The maximal quantity of AmB encapsulated, reported to 100 mg of the microspheres, was 7.2 mg with an encapsulation ratio of 60%.

Hetal Paresh Thakkar *et al.*,[17] (2008), in their work "**Effect of crosslinking agent on the characteristics of Celecoxib loaded chitosan microspheres**" reported that chitosan microspheres were prepared by emulsification cross linking method. The entrapment efficiency of glutaraldehyde and formaldehyde cross-linked microspheres were significantly higher ($p<0.05$) than heat cross-linked microspheres. *In-vitro* drug release studies indicated that the microspheres cross linked using gluteraldehyde showed slower release rate than those cross linked with formaldehyde while the heat cross-linked microspheres showed the fastest release.

M.Nappinai. *et al.*,[18] (2007), in their work "**Formulation and evaluation of microspheres of Diltiazem hydrochloride"** reported that microspheres of diltiazem hydrochloride were formulated using combination of poly ethylene glycol 6000 and eudragit RS 100 and eudragit RS 100 alone by solvent evaporation

and non solvent addition methods with an aim to prolong its action. Formulation prepared using the combination of the retardants exhibited first order of drug release and zero order for preparation containing eudrajit RS alone.

A.Mukherjee *et al.*,[19] (2007), in their work "**Preparation and characterization of poly-ε-caprolactone particles for controlled Insulin delivery"** reported that the method was for the efficient encapsulation of insulin in poly-ε-caprolactone microspheres and nanospheres using a water-in-oil-in-water double emulsion solvent evaporation method. The microspheres and nanospheres formed were characterized for entrapment efficiency, percentage yield, particle size analysis, morphological characteristics and the drug release profiles. The studies revealed a successful formulation of smooth spherical poly-ε-caprolactone microspheres and nanospheres encapsulating insulin, thus highlighting them as potential controlled drug delivery systems.

D.M Morkhade *et al.*,[20] (2007), in their work **" Evaluation of gum dammar as a novel microencapsulating material for Ibuprofen and Diltiazem hydrochloride"** reported that microparticles were prepared by oil-in-oil emulsion solvent evaporation method. The effect of different gum : drug ratios and solubility of drug on microparticle properties was principally investigated. With diltiazem hydrochloride, gum dammar produced

bigger (40-50μm) and fast drug releasing microparticles with low encapsulation efficiencies(44-57%). Contrary, with ibuprofen, gum dammar produced small (24-33μm) microparticles with better drug encapsulation (85-91%) and sustained drug delivery. The increase in gum: drug ratio had shown an increase in particle size, encapsulation efficiency and decrease in drug release rate in all cases.

Shaobing Wang *et al.*,[21] (2007), in their work **"Disodium norcantharidate loaded poly(ε-caprolactone) microspheres: preparation and evaluation"** reported that Poly(ε-caprolactone) (PCL) microspheres encapsulating disodium norcantharidate (DSNC), a drug in salt form and with high water solubility, were prepared by s/o/w solvent evaporation technique and characterized in terms of size, morphology, encapsulation efficiency and drug release. The viscosity of s/o dispersion was crucial to the successful encapsulation of DSNC. Scanning electron microscopy (SEM) studies had shown that the drug-loaded microspheres had coarse surface and porous internal structure. The analysis of X-ray diffraction (XRD) indicated that there was no interaction between DSNC and PCL, but the degree of crystallinity of PCL decreased with the introduction of the drug. The drug release profiles indicated an initial burst release followed by a slow release, and a further investigation into the release mechanism implied that the

release of DSNC from PCL microspheres was caused by a combination of diffusion and osmotic pressure.

Mundargi R.C *et al.*,[22] (2007), in their work **"Development and evaluation of novel biodegradable microspheres based on poly(d,l-lactide-co-glycolide) and poly(epsilon-caprolactone) for controlled delivery of doxycycline in the treatment of human periodontal pocket: *in-vitro* and *in-vivo* studies"** reported that development of novel biodegradable microspheres prepared by water-in-oil-water (W/O/W) double emulsion technique using the blends of poly(d,l-lactide-co-glycolide) (PLGA) and poly(epsilon-caprolactone) (PCL) in different ratios for the controlled delivery of doxycycline (DXY). Doxycycline encapsulation of up to 24% was achieved within the polymeric microspheres. Blend placebo microspheres, drug-loaded microspheres and pristine DXY were analyzed by Fourier transform FT-IR, which indicated no interaction between drug and polymers. DSC on drug-loaded microspheres confirmed the polymorphism of DXY. SEM confirmed the spherical nature and smooth surfaces of the microspheres produced. *In-vitro* release studies performed in 7.4 pH media indicated the release of DXY from 7 to 11 days, depending upon the blend ratio of the matrix. Up to 11 days, DXY concentrations in the gingival crevicular fluid were higher than the minimum inhibitory concentration of DXY against most of the periodontal pathogens.

One of the developed formulations was subjected to in vivo efficacy studies in thirty sites of human periodontal pockets.

Xudong Wang *et al.*,[23] (2006), in their work **"Drug distribution within poly (ε-caprolactone) microspheres and *in vitro* release"** reported that Poly(ε-caprolactone) (PCL) microspheres loaded with two model compounds (*p*-nitroaniline and rhodamine B) with different water solubilities were prepared by an s/o/w single emulsion solvent evaporation method. The microspheres morphology were investigated by SEM, drug loading and encapsulation efficiency were also calculated. Drug distribution within microsphere matrix was studied by confocal laser scanning microscopy. *p*-Nitroaniline, as a more hydrophobic compound, distributed more evenly in the matrix, while the more hydrophilic compound rhodamine distributed close to the surfaces of microspheres. The *in-vitro* release profiles therefore were different. This study helps to further understand the drug release mechanism from microsphere matrix, and design effective long-term drug delivery system.

Bhalero S.S *et al.*,l[24] (2003), in their work **"Study of processing parameters influencing the properties of Diltiazem hydrochloride microspheres"** reported that prepared diltiazem hydrochloride- ethyl cellulose microspheres by water- in- oil emulsion solvent evaporation technique produced small and

spherical microspheres having a mean microsphere diameter in the range of 40-300µm and entrapment efficiency of 60-90% were obtained. The *in-vitro* release profile could be altered significantly by changing various processing parameters to give a controlled release of drug from the microspheres. The stability studies of the drug- loaded microspheres showed that drug was stable at storage temperatures, 5-55˚C, for 12 weeks.

J.L.Maia *et al.*,[25] (2003), in their work **"The effect of some processing conditions on the characteristics of biodegradable microspheres obtained by emulsion solvent evaporation process"** reported that unloaded microspheres were prepared from polyhydroxybutyrate (PHB) and polyhydroxybutyrate-co-valerate (PHB-HV) polymers using an oil-in-water emulsion solvent evaporation method. The study was conducted to evaluate how the polymer and some process parameters affect properties of the final microspheres such as particle size, superficial area, zeta potential, surface morphology and microsphere degradation. The variables included surfactant concentration in the emulsion water phase and solvent composition. From the results, it was found that the parameters affecting microsphere size the most were surfactant concentration in the emulsion's water phase and solvent composition. Properties such as zeta potential, surface area and surface morphology remained pratically unchanged over the range

of the processing conditions studied here.

B.K.Kim *et al.*,[26] (2003), in their work **"Characteristics of Felodipine- loaded poly (ε-caprolactone microspheres"** reported that Felodipine-loaded poly (ε-caprolactone) microspheres were prepared by two methods that is, the conventional emulsion solvent evaporation method and the quenching method. The results show that, when conventional emulsion solvent evaporation method was u sed, the o/w-method produced smaller mean size and higher encapsulation efficiency compared with the w/o-method. The encapsulation efficiencies increased with an increase in the molecular weight and a decrease in crystallinity of PCL. The size of microspheres varied with the type of emulsion stabilizer used, smaller microspheres with PVA and narrow size distribution with Pol 237. When water-soluble solvents such as acetonitrile and ethyl formate were used, the encapsulation efficiencies decreased due to higher evapouration rate. When quenching methods were used, in contrast to the conventional emulsion solvent evapouration method, very narrow size-distributed but larger microspheres were obtained.

Sang – wook Sun *et al.*,[27] (2003), in their work **"Surfactant free microspheres of poly(ε- caprolactone)/poly (ethylene glycol)/poly(ε-caprolactone) triblock copolymers as a**

protein carrier" reported that a poly(ε-caprolactone)/ poly(ethylene glycol)/poly(ε-caprolactone) (CEC) triblock copolymer was synthesized by the ring-opening of ε-caprolactone with dihydroxy poly (ethylene glycol) to prepare surfactant-free microspheres. When dichloromethane (DCM) or ethyl formate (EF) was used as a solvent, the formation of microspheres did not occur. Although the microspheres could be formed prior to lyophilization under certain conditions, the morphology of microspheres was not maintained during the filtration and lyophilization process. Surfactant-free microspheres were only formed when ethyl acetate (EA) was used as the organic solvent and showed good spherical microspheres although the surfaces appeared irregular. The content of the protein in the microsphere was lower than expected, probably because of the presence of water channels and pores. The protein release kinetics showed a burst release until 2 days and after that sustained release pattern was showed.

D.Vijaya Ramesh *et al.*,[28] (2002), in their work " **Microencapsulation of FTIC- BSA into poly(ε- caprolactone) by water- in -oil –in-oil solvent evaporation technique***"* reported the encapsulation of protein into poly (ε-caprolactone) microspheres. The preparation procedures of microspheres preparation were with an aim to get different particle size by changing the preparative variables such as polymer concentration, volume of internal aqueous phase, homogenization speed and

stirring speed of solvent evaporation. The morphological characteristics of the particles and release profiles of the labeled protein were analysed. In optimum conditions spherical and smooth PCL microspheres were obtained with high encapsulation efficiency. The particle size were reduced as the concentration of the polymer solution reduced. The homogenization speed does not show any effect on particle size and entrapment characters. The release of FITC-BSA lasted longer as the particle size increased.

Aberturas M.R *et al.*,[29] (2002), in their work **"Development of a new cyclosporine formulation based on poly(caprolactone) microspheres"** reported that the study describes the development of a new cyclosporine formulation based on polycaprolactone (PCL) microspheres (MS) prepared by the solvent evaporation method. Ternary phase diagrams were used to identify the domains where MS were formed. The application of central composite designs established the influence of several technological (stirring speed) and formulation factors (polymer and surfactant amounts, and organic solvent volume) on the size of PCL MS. The experimental design had shown that the stirring speed and the organic phase volume were the only parameters significantly affecting the MS size. Experimental conditions selected to obtain CyA-loaded MS of 2.5 μm resulted in a high entrapment percentage (98.4 ± 0.66%), with the drug dissolved or molecularly dispersed within the dense polymeric matrix of MS. After 12

months of storage at 8 °C and RT, PCL MS remained physically stable, although the crystallinity of the polymer increased by 35% upon storage at both temperatures. Freeze-drying studies revealed that MS could be successfully lyophilized in the absence of cryoprotectants without significant changes of the drug entrapment. Therefore, a stable MS-based CyA formulation was easily prepared and characterized. This formulation offer the possibility of CyA administration through different routes.

Arica B *et al.*,[30] (2002), in their work **"Biodegradable bromocryptine mesylate microspheres prepared by a solvent evaporation technique. I: Evaluation of formulation variables on microspheres characteristics for brain delivery"** reported that the effect of formulation parameters (e.g. polymer, emulsifying agent type and concentration) on the characteristics of the microspheres produced, the efficiency of drug encapsulation, the particle size distribution and *in-vitro* drug release rates from the bromocryptine mesylate microspheres were investigated using a 3(2) factorial design. Bromocryptine mesylate was encapsulated into biodegradable polymers using the following three different polymers; poly(L-lactide), poly(D,L-lactide) and poly(D,L-lactide-co-glycolide). The SEM photomicrographs had shown that the morphology of the microspheres greatly depended on the polymer and emulsifying agent. The results indicate that, regardless of the

polymer type, increase in emulsifying agent concentration from 0.25-0.75% w/v markedly decreases the particle size of the microspheres. Determination of particle size revealed that the use of 0.75% w/v of emulsifying agent concentration and a polymer solution concentration of 10% w/v resulted in optimum particle size. Polymer type has a less pronounced effect on the percentage encapsulation efficiency and particle size of microspheres.

Tomaz Kriczka *et al.*,[31] (1999), in their work **"Kinetics of a nucleoside release from lactone-caprolactone and lactide-glycolide polymers *in vitro*"** reported that the rate of release of a model nucleoside (adenosine, 5%, w/w) from nine different lactide-glycolide or lactide-caprolactone polymers. The polymer discs were eluted every second day with an artificial cerebrospinal fluid at the elution rate roughly approximating the brain extracellular fluid formation rate. Adenosine in eluate samples were assayed by HPLC. Three polymers exhibited a relatively constant release of adenosine for over four weeks, resulting in micromolar concentrations of nucleoside in the eluate. This points to the neccessity of further development of polymers of this types as intracerebral nucleoside delivery systems for local treatment of brain tumors.

M.A.Bayomi *et al.*,[32] (1998), in their work **" Preparation of**

casein- chitosan microspheres containing Diltiazem hydrochloride by an aqueous coacervation technique" reported that Sustained release microspheres were prepared with colloidal coacervation technique in a completely aqueous environment. The entrapment efficiencies of the microspheres were variables (14.5–53.7%) and depends on the preparation conditions.. The dissolution profiles of drug from casein–chitosan microspheres showed retarded release pattern of the drug in distilled water. Casein and chitosan concentrations, initial drug concentration and stirring time were found to be the main parameters that affect the properties and the performance of the prepared microspheres. The retarded release of DTZ was increased by increasing casein concentration, and stirring time. On the other hand, increasing chitosan concentration and using high initial drug loading showed a fast drug release.

SCOPE OF THE WORK

Treatment for an ailment by the physician mostly involved drug substances. The use of drug substances had become inevitable in the modern days. The major problem faced by the patients in taking the medications are to be overcome by altering the design of dosage form or properties of the drug moiety.

The scope of any formulation primarily focuses on safety and efficacy of the drug delivery system. Now the focus has been slightly moved to the patient's convenience and acceptance, where still the safety and efficacy remain integrated with design.

There are many disorders and diseases that can be treated to obtain the better patient outcomes only when the drugs are being properly taken. Eg: Diabetes, hypertension requires regular monitoring of the respective parameters. The patients find difficult being fully compliance to the given prescription. The reasons may vary from mainly the frequency of dosing, ease of administration including route etc.

Some of the drugs may not be available in the therapeutic level or not well absorbed (low availability or eliminated rapidly from the body)and those drugs can be comfortably converted to a sustained release or controlled release drug delivery system to provide a better patient comfort in terms of acceptance and convenience.

The previous section of this discussion (introduction and literature survey) had given us a deep insight of the advantages, disadvantages and design of the controlled drug delivery systems. It also gives on the possibility of converting diltiazem hydrochloride (model drug) into a controlled release drug delivery system. The drug has poor bioavailability (30%-40%) which in because of large amount of drugs undergoes first pass metabolism. The bioavailability of the drug can be increased by converting into the CRDDS by using poly (ε- caprolactone) a naturally obtained biodegradable and biocompatible substances.

OBJECTIVE OF THE WORK

The main objective of any drug therapy in CRDDS is achieving a desired concentration of the drug in plasma or tissue which is therapeutically effective for an extended period of time. Controlled release system as rapidly emerged over past three decades as a new discipline in formulation that offers novel approaches to microparticulate dosage forms for bio-active agents, so far natural and synthetic polymers were employed to obtain control over release pattern of drugs.

Diltiazem hydrochloride is a calcium channel blocker, which is mainly used in the management of angina pectoris. It was used in the present work since it has low bioavailability (30-40%), a dose of 30 mg to be taken thrice a day, it has low plasma half life of 3.5 hr, and undergoes extensive first pass metabolism. To avoid these problem this drug is formulated into sustained release or controlled release dosage forms, which will release the drugs slowly into the GIT and maintain the constant drug concentration of the drug into the plasma for longer period of time.

The use of biodegradable polymers as drug carriers is one of the main objectives of recent researchers dealing with long acting dosage forms. Of the various biodegradable polymers used for the development of sustained release formulations, poly (ε-caprolactone) has been reported to be advantageous since they are

biocompatible. PCL is prepared by ring opening of ε-caprolactone using a catalyst such as stannous octanoate. Poly (ε-caprolactone) is aliphatic polyester polymer, suitable for controlled drug delivery due to high permeability to many drugs and at the same time being from toxicity.

The basic goal of current study is to develop poly(ε-caprolactone) microspheres containing the drug diltiazem hydrochloride. The poly(ε-caprolactone) microspheres were formulated and evaluated with respect to variables like change in stirring speed and amount of polymer used. The delivery system designed for an oral route of administration where a considerable research had been done. Such studies had reported to achieve releases either completely or partially.

So the current study aimed at design of an oral controlled release drug delivery system (micropsheres) for diltiazem hydrochloride using poly(ε-caprolactone).

PLAN OF WORK

The work entitled "**Formulation and Evaluation of Diltiazem hydrochloride microcapsules for oral controlled release drug delivery using poly (ε- caprolactone)**" was planned and carried out for a period of 9 months (May 2009- January 2010) in the following manner.

Phase I : Literature survey

(May 2009) Design of the study

Phase II : Preparation of the standard graphs

(June -Aug 2009) Preparation of diltiazem hydrochloride microspheres using poly (ε-caprolactone)

Phase III : **Evaluation of the prepared microspheres**

(Sept.-Dec. 2009)

- Compatibility studies using IR spectrophotometer
- Physical characterization by particle size analysis

❖ Scanning electron microscope

❖ Optical microscope

- Drug content analysis

❖ Actual drug loaded into the microspheres

❖ *in-vitro* drug analysis of microspheres

Phase IV : Data analysis and project submission.

(Jan. 2010)

MATERIALS USED

MATERIALS

Name of the materials	Name of the company
Diltiazem hydrochloride(gift sample)	Micro labs Ltd, Bangalore
Poly (ε- caprolactone) ca 60000	Hi media biosciences Ltd, Mumbai.
Light liquid paraffin LR	Spectrum reagents and chemicals Pvt. Ltd. Edwar, Aluva
Tween -80 LR	Indian research products , Mumbai
Dichloromethane LR	Qualigens Fine chemicals Ltd Mumbai
Sodium hydroxide AR	S .D Fine chemicals Ltd, Mumbai
Potassium dihydrogen monophosphate AR	S.D.Fine chemicals Ltd, Mumbai

EQUIPMENTS

Name of equipment	Name of the company
Optical Microscope and stage Micrometer	Erma. Japan
Remi hi-speed motor	Universal motors. Mumbai
UV / Vis Spectrophotometer	JASCO V-530.
Dissolution apparatus	Electrolab TDT-08L. Chennai.
Scanning Electron Microscope	JSM6400
Digital balance	Denver instruments
IR Spectrophotometer	Jasco-FT-IR 8201 PC
pH tester 1 (water proof)	Oakton instruments.

METHODOLOGY

PREPARATION OF STANDARD GRAPH FOR DILTIAZEM HYDROCHLORIDE

Preparation of stock solution[39]

100 mg of diltiazem hydrochloride was dissolved in phosphate buffer pH 7.4 and the volume was made upto 100 ml in a volumetric flask by using phosphate buffer to give **1 mg/ml** concentration.

1ml of the above solution was taken and volume made upto 100ml by phosphate buffer to give 10µg/ml concentration which will be used as a stock solution. The details were given in **Table No:3.**

Preparation of standard solution

Table No : 3 Standard solution details

Test tube no	Stock solution (10µg/ml) ml	Phosphate buffer ml	Concentration (µg/ml)
1	0	10	0
2	1	9	1
3	2	8	2
4	3	7	3
5	4	6	4
6	5	5	5
7	6	4	6

8	7	3	7
9	8	2	8
10	9	1	9
11	10	0	10

The absorbance of the above solutions were measured at 236.5nm (λmax) checked using UV spectrophotometer. Phosphate buffer is used as blank.

PREPARATION OF POLY(ε-CAPROLACTONE) MICROSPHERES

Poly (ε-caprolactone) microspheres were prepared by solvent evaporation technique. Accurately weighed quantity of poly (ε-caprolactone) was dissolved in 10 ml of dichloromethane, then 200mg of diltiazem hydrochloride was dissolved in this polymer phase. This solution was poured in 100ml of liquid paraffin containing the emulsifier tween 80 and continuously stirred for 5 hours. The microspheres were filtered and washed three times with 50ml of n-hexane and dried at room temperature for 12 hours. Microspheres dried at room temperature were then weighed and the yield of microspheres prepared were calculated. The variables used in this experiment like drug/polymer ratios, striring speed ying concentrations of emulsifier and varvaried as given in **Table No.4.**

Table No : 4 Composition of different batches of microspheres

SL No	Formulation code	Drug (mg)	Polymer (mg)	% tween 80 in liquid paraffin	Stirring speed	Drug to polymer ratio
1	F1	200	200	1.3	500	1:1
2	F2	200	400	1.3	500	1:2
3	F3	200	600	1.3	500	1:3
4	F4	200	8 00	1.3	500	1:4
5	F5	200	1000	1.3	500	1:5
6	F6	200	200	1.3	1000	1:1
7	F7	200	400	1.3	1000	1:2
8	F8	200	600	1.3	1000	1:3
9	F9	200	800	1.3	1000	1:4
10	F10	200	1000	1.3	1000	1:5
11	F11	200	200	1.3	1500	1:1
12	F12	200	400	1.3	1500	1:2
13	F13	200	600	1.3	1500	1:3
14	F14	200	800	1.3	1500	1:4
15	F15	200	1000	1.3	1500	1:5
16	F16	200	200	1.95	500	1:1
17	F17	200	400	1.95	500	1:2
18	F18	200	600	1.95	500	1:3
19	F19	200	800	1.95	500	1:4
20	F20	200	1000	1.95	500	1:5
21	F21	200	200	0.65	500	1:1
22	F22	200	400	0.65	500	1:2
23	F23	200	600	0.65	500	1:3
24	F24	200	800	0.65	500	1:4
25	F25	200	1000	0.65	500	1:5
26	F26	200	200	0.65	1000	1:1
27	F27	200	400	0.65	1000	1:2
28	F28	200	600	0.65	1000	1:3
29	F29	200	800	0.65	1000	1:4
30	F30	200	1000	0.65	1000	1:5

EVALUATION OF PREPARED MICROSPHERES

COMPATIBILITY STUDIES

IR spectral analysis[40]

Weighed amount of the drug (3mg) was mixed with 100mg of potassium bromide (dried at 40-50˚C), which was then compressed under 10 tonn pressure in a hydraulic press to form a pellet was then scanned from 4000-400 cm 1 in IR spectrophotometer. The same procedure was repeated for various formulations prepared.

The IR spectrum of diltiazem hydrochloride drug was compared with IR spectrum of the microspheres to understand the compatibility.

PHYSICAL CHARACTERIZATION

Particle size analysis

- **Scanning Electron Microscope**

The surface morphology and texture of microcapsules prepared were studied by subjecting the samples using scanning electron microscope (SEM). SEM photographs were taken on a JSM6400 scanning electron microscope at magnification ranging 210 to 1700X at room temperature. The microcapsules were sputtered with gold to make the surface conductive before scanning.

❖ **Optical microscopic method[41]**

The particle size distribution analysis was performed by using an optical microscope. In this method a slide containing a sample of microspheres were dispersed uniformly in liquid paraffin and was mounted to assess the particle size distribution by using an eye piece micrometer which was calibrated as per the given procedure.

Standardization of eye piece micrometer[42]

Calibrate the eye piece micrometer with the help of the stage micrometer (standard). Note the division of the eye piece micrometer scale and stage micrometer scale which coincide with each other, use the following formula to assess how much is one division of eye piece micrometer.

One division of stage micrometer= 10μ

One division of eye piece micrometer

$$= \frac{\text{Number of division of stage micrometer}}{\text{Number of division of eye piece micrometer}} \times 100$$

After obtaining the required data like frequency of particle in each size range (number distribution), various statistical equivalent diameters are calculated using the following equations given below and in **Table No: 5**

$$d\,\text{mean} = \left(\frac{\sum nd^{p+f}}{\sum nd^{f}} \right)^{1/p}$$

n = number of particles in a size range in μm

d = midpoint in a size range

p = an index related to size of an individual particle represents

if p = 1,2,3 represents length, surface, volume respectively

p = positive- arithmetic mean

p = negative- arithmetic mean

p = zero- geometric mean

f = 0,1,2,

Table No : 5 Statistical Equivalent diameters[41]

$\left(\frac{\sum nd^{p+f}}{\sum nd^{f}}\right)^{1/}$	**P**	**f**	**Type of mean**	**Size parameter**	**Frequency**	**Mean Diameter**
$\left(\frac{\sum nd}{\sum n}\right)$	1	0	Arithmetic	Length	Number	Length – number mean d_{in}
$\sqrt{\frac{\sum nd^2}{\sum n}}$	2	0	Arithmetic	Surface	Number	Surface – number mean d_{sn}
$\sqrt[3]{\frac{\sum nd^3}{\sum n}}$	3	0	Arithmetic	Volume	Number	Volume– number mean d_{vn}
$\frac{\sum nd^3}{\sum nd^2}$	1	2	Arithmetic	Length	Surface	Volume – Surface or surface – weighted mean, d_{vs}

DRUG CONTENT ANALYSIS

The amount of drug loaded was assessed by the following methods

- Drug actually loaded into the microspheres
- *In-vitro* drug release studies

Drug actually loaded

100 mg of diltiazem hydrochloride loaded poly (ε-caprolactone) microspheres were crushed with 50ml of phosphate buffer. After suitable dilution the absorbance was measured UV/Vis spectrophotometer at 236.5nm. Amount of diltiazem hydrochloride actually loaded into the microspheres was estimated with the help of a standard graph prepared using phosphate buffer as blank.

***In-vitro* drug release studies**

Microspheres were kept in an empty hard gelatin capsule shell and into apparatus for assessing the drug release pattern. Diltiazem hydrochloride release from poly (ε- caprolactone) microspheres were investigated in phosphate buffer solution pH 7.4, using USP dissolution apparatus II, the amount of drug release from the microspheres were measured spectrophotometrically at 236.5nm using phosphate buffer pH 7.4 as blank.

100 mg of microspheres taken in an empty hard gelatin capsule shell was added to 900 ml of phosphate buffer solution in

the dissolution apparatus and rotates at 100 rpm/min, the temperature of the medium maintained at 37 ± 5˚C for 8 hrs. Aliquots of 5ml were withdrawn at specific intervals like 0hr, .5,1,2,3,4,5,6,7 and 8 hr and analyzed spectrophotometrically at 236.5nm after suitable dilution if required. The *in-vitro* drug release profile obtained by plotting percentage release versus time in hours was also done.

DISSOLUTION KINETICS OF DRUG RELEASE [43,44]

To study the release kinetics, data obtained from *in-vitro* drug release studies were plotted in various kinetic models: **Zero order** (Equation 1) as cumulative amount of drug released vs time, **First order** (Equation 2) as log cumulative percentage of drug remaining vs time, **Higuchi's model** (Equation 3) as cumulative percentage of drug released vs square root of time and **Korsmeyer's** (Equation 4) log cumulative percentage of drug released vs. log time

Zero order

$$\mathbf{C = K_0\, t.} \qquad \text{(Equation1)}$$

Where K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration vs time would yield a straight line with a slope equal to K_0 and intercept the origin of the axes.

First order

$$\mathbf{LogC = LogC_o - kt/2{:}303} \qquad \text{(Equation2)}$$

Where C_0 is the initial concentration of drug, k is the first order constant, and t is the time.

Higuchi's

$$\mathbf{Q = Kt^{1/2}} \qquad \text{(Equation3)}$$

Where K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time.

Drug release were plotted in Korsmeyer et al's equation (Equation 4) as log cumulative percentage of drug released vs log time, and the exponent n was calculated through the slope of the straight line.

Korsmeyer's

$$\mathbf{M_{t/M\infty} = Kt^n} \qquad \text{(Equation4)}$$

Where M_t/M_∞ is the fractional solute release, t is the release time, K is a kinetic constant.

RESULTS AND DISCUSSION

PREPARATION OF STANDARD GRAPHS

An attempt was made to confirm the reported λ_{max} values by scanning the solutions prepared with different solvents of stock solution and the lambda max(λ_{max}) was found as 236.5nm in phosphate buffer (pH 7.4).

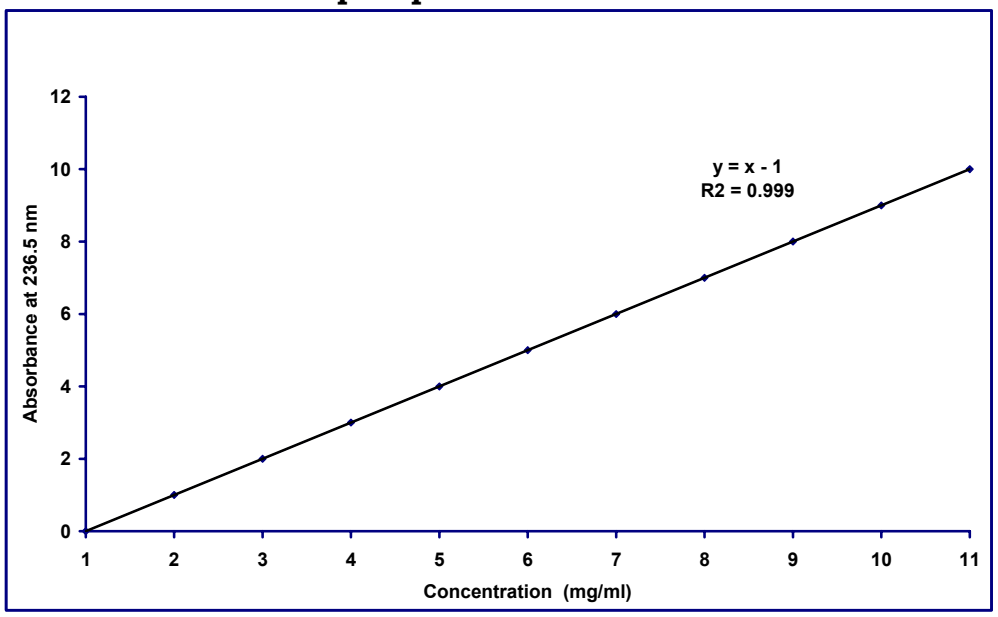
The standard graph for the purpose of estimating the amount of drug loaded into the poly (ε-caprolactone) microspheres and to assess the amount of drug release in the *in-vitro* drug release samples were done according to the procedure explained in the methodology. The results obtained were given in the **Table No: 6** and **Figure.No: 4.**

Table No : 6 Absorbance of Diltiazem hydrochloride in phosphate buffer (pH 7.4)

S.No	Concentration (µg/ml)	Absorbance at 236.5 nm
1	0.0	0.0
2	1.0	0.2022
3	2.0	0.3103
4	3.0	0.4681
5	4.0	0.6410
6	5.0	0.8136
7	6.0	0.9498
8	7.0	1.0911
9	8.0	1.2899

10	9.0	1.4176
11	10.0	1.5827

Figure 4 : Standard graph of diltiazem hydrochloride with phosphate buffer 7.4



COMPATIBILITY STUDIES

IR spectral analysis

The microspheres were prepared as discussed earlier in methodology with different variables like drug to polymer ratio, stirring speed and changing concentration of emulsifier.

The obtained micropsheres were subjected to compatibility studies by using an IR spectrophotometer. The IR spectrum obtained for DTZ, was compared with the IR spectrum of various formulations prepared. The presence of characteristic peaks at 3032.51cm^{-1}, 1373.07cm^{-1}, 1743.33cm^{-1}, 1679.69cm^{-1},

689.427cm^{-1} and 1607.38cm^{-1} representing the DTZ were also found to present in the spectrum of different formulations.

The above discussion confirmed that the drug taken was not having any interactions with the polymer used. Hence the polymer poly (ε-caprolactone) was found to be compatible with the drug (DTZ). To confirm any effect on increasing concentration of polymer with drug different batches were studied and reports revealed that the increase in concentration does not produce any incompatibility problem between polymer and drug.

The IR spectrum of the individual drug, polymer and for selected formulations were given in the following **Figure.No: 5-10**.

Fig.No. 5: IR Sepctrum of Diltiazem Hydrochloride

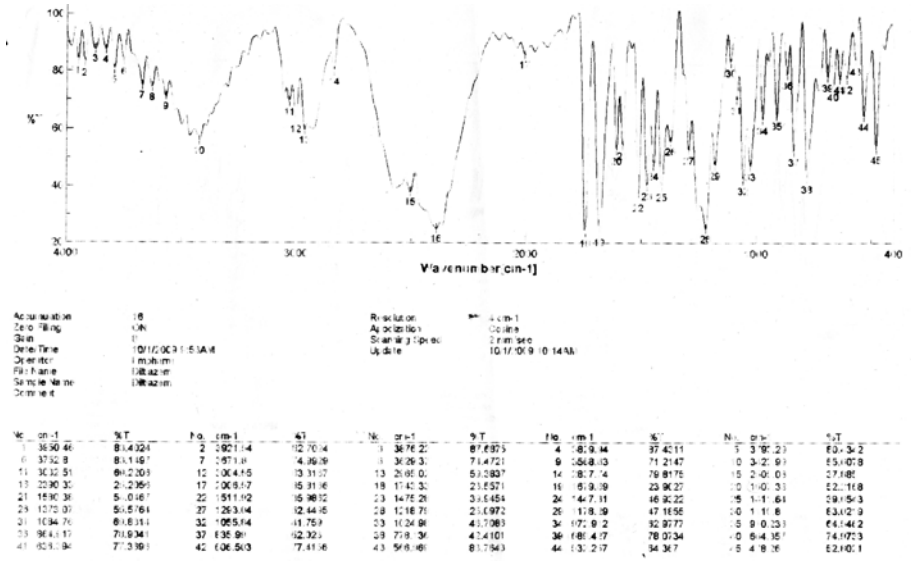
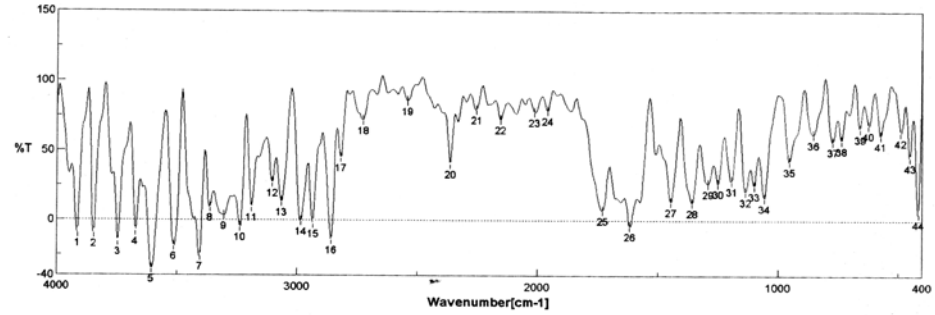


Fig.No. 6 : IR spectrum of F_3



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File Name	F8		
Sample Name	F8		
Comment			

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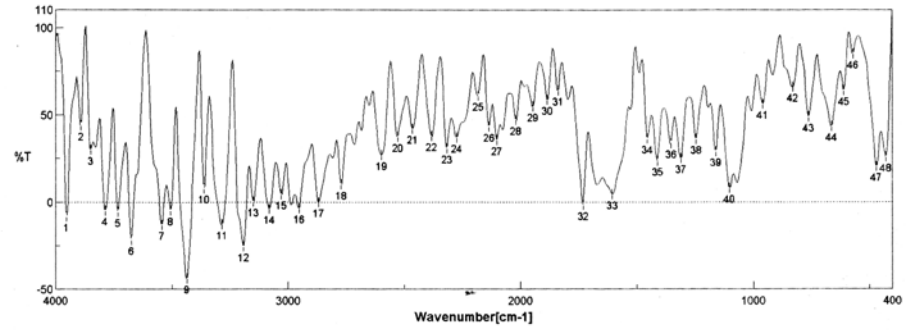
1	3914.79	-9.29872	2	3845.36	-9.70166	3	3745.08	-15.5296	4	3671.8	-8.01287	5	3606.23	-35.0069
6	3513.67	-18.6154	7	3405.67	-25.9347	8	3363.25	9.19516	9	3305.39	3.26995	10	3239.82	-4.84792
11	3189.68	9.01033	12	3104.83	26.7679	13	3066.26	13.0198	14	2985.27	-0.80731	15	2935.13	-2.55618
16	2857.99	-14.0401	17	2819.42	45.0366	18	2726.85	71.6775	19	2541.72	85.844	20	2364.3	40.4458
21	2256.31	79.3875	22	2156.03	72.3384	23	2013.32	77.6905	24	1959.32	78.6502	25	1727.91	7.07766
26	1616.06	-4.33386	27	1446.35	13.398	28	1357.64	12.662	29	1292.07	26.5217	30	1249.65	26.6117
31	1191.79	28.5261	32	1133.94	20.8201	33	1099.23	24.8148	34	1056.8	16.29	35	952.663	42.6876
36	852.382	61.9368	37	771.387	56.8966	38	736.674	57.8033	39	659.536	66.1858	40	624.823	68.5974
41	574.683	61.1676	42	489.831	63.3527	43	451.261	45.2471	44	416.549	4.83283			

Fig.No. 7 : IR spectrum of F_8



Accumulation	16	Resolution	16 cm-1
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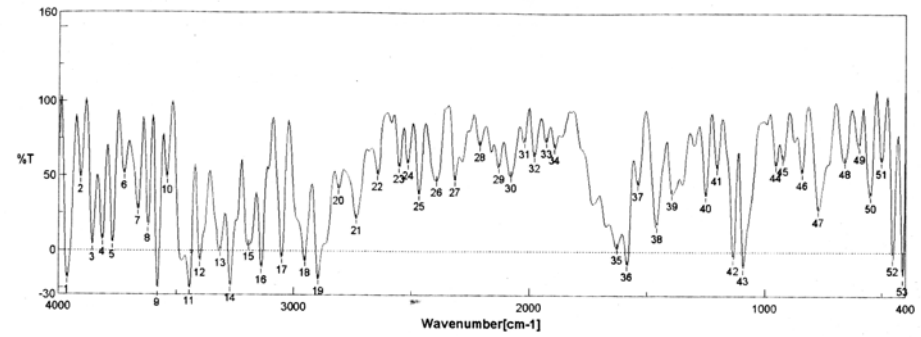
Operator	C.Geetha		
File Name	F18		
Sample Name	F18		
Comment			

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3953.36	-8.3192	2	3891.65	43.7947	3	3849.22	29.9339	4	3787.51	-4.87867	5	3733.51	-5.74125
6	3675.66	-21.4643	7	3544.52	-12.884	8	3505.95	-4.87288	9	3436.53	-43.7797	10	3363.25	7.96981
11	3286.11	-12.795	12	3193.54	-25.5676	13	3151.11	0.244641	14	3081.69	-3.85174	15	3031.55	4.49546
16	2954.41	-3.58725	17	2869.56	0.0111187	18	2773.14	10.0572	19	2599.57	26.7862	20	2530.15	37.3825
21	2464.58	41.9042	22	2383.59	37.7289	23	2318.02	30.8748	24	2275.59	37.0927	25	2183.02	60.9981
26	2136.74	43.1215	27	2102.03	35.3674	28	2021.03	47.0444	29	1947.75	54.5952	30	1886.04	58.4909
31	1839.76	63.818	32	1731.76	-1.65799	33	1604.48	4.43194	34	1454.06	36.227	35	1411.64	23.6561
36	1353.78	33.6768	37	1311.36	25.183	38	1245.79	35.9357	39	1160.94	28.9867	40	1103.08	7.97576
41	960.377	56.2506	42	829.241	65.79	43	759.816	48.645	44	663.393	43.3519	45	609.396	63.8767
46	570.826	84.8511	47	470.546	20.2823	48	428.12	25.7939						

Fig.No. 8: IR spectrum of F_{18}



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Operator	C.Geetha		
File Name	F3		
Sample Name	F3		
Comment			

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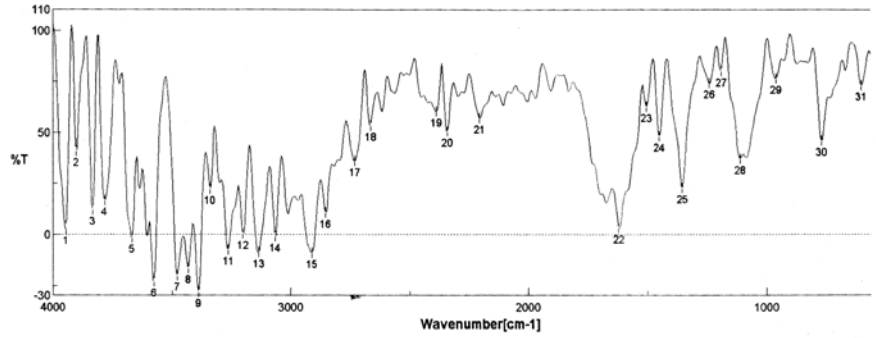
1	3964.93	-19.1361	2	3907.07	48.4384	3	3856.93	2.75275	4	3814.51	6.44199	5	3772.08	4.41102
6	3721.94	50.989	7	3664.09	27.2401	8	3621.66	15.5237	9	3579.23	-27.5458	10	3540.67	48.4094
11	3444.24	-26.3134	12	3401.82	-7.57282	13	3313.11	-0.943554	14	3270.68	-24.3139	15	3193.54	3.3499
16	3139.54	-11.9234	17	3050.83	-5.58714	18	2954.41	-7.25472	19	2896.56	-19.6293	20	2811.7	41.2831
21	2734.57	21.4308	22	2645.86	50.8443	23	2553.29	56.1119	24	2514.72	58.4172	25	2468.44	37.7174
26	2395.16	47.2796	27	2318.02	47.3683	28	2210.02	70.6966	29	2129.03	55.886	30	2078.89	50.2146
31	2021.03	73.0147	32	1978.61	63.5949	33	1928.47	73.1374	34	1893.75	69.1605	35	1827.63	2.03166
36	1585.2	-9.23656	37	1538.92	44.7452	38	1457.92	16.3056	39	1392.35	38.5079	40	1249.65	37.19
41	1203.36	55.3259	42	1133.94	-5.22064	43	1091.51	-11.7873	44	952.663	57.7879	45	921.807	61.8783
46	840.812	52.4559	47	771.387	27.5841	48	659.536	60.2579	49	597.825	70.1059	50	547.685	37.0352
51	501.401	60.2988	52	455.118	-5.11497	53	412.692	-18.5221						

Fig.No. 9: IR spectrum of F_{23}



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Operator	C.Geetha		
File Name	F28		
Sample Name	F28		
Comment			

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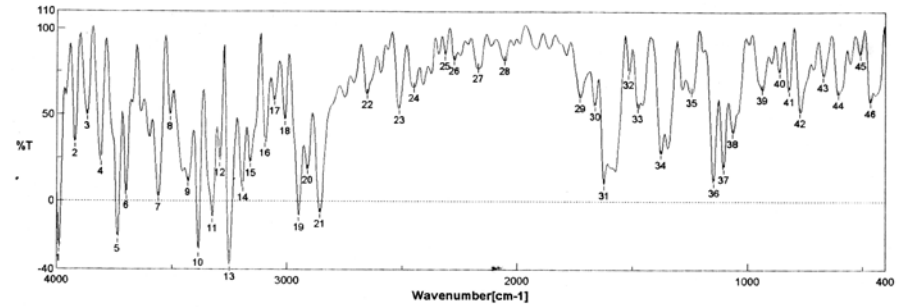
1	3945.64	2.50703	2	3903.22	40.9484	3	3833.79	12.083	4	3783.65	16.7376	5	3667.94	-1.620
6	3575.36	-23.1026	7	3478.95	-20.2767	8	3432.67	-16.5896	9	3390.24	-28.2323	10	3340.1	22.303
11	3262.97	-7.37425	12	3201.26	0.179106	13	3135.69	-8.68231	14	3066.26	-0.906541	15	2911.99	-8.995
16	2854.13	10.6611	17	2730.71	35.8779	18	2665.14	53.3741	19	2387.44	60.3026	20	2341.16	50.413
21	2206.17	56.8648	22	1619.91	3.03508	23	1504.2	62.8258	24	1450.21	48.0372	25	1353.78	22.714
26	1238.08	74.218	27	1187.94	80.0929	28	1110.8	37.0859	29	960.377	76.2984	30	767.53	45.778
31	601.682	73.0534	32	501.401	64.2735	33	428.12	41.8928						

Fig.No. 10: IR spectrum of F_{28}



Accumulation	16	Resolution	16 cm-1
Zero Filling	ON	Apodization	Cosine
Gain	128	Scanning Speed	2 mm/sec
Date/Time	1/8/2010 3:24PM	Update	1/12/2010 11:48AM
Operator	C.Geetha		
File Name	F23		
Sample Name	F23		
Comment			

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3991.93	-27.4849	2	3922.5	33.1105	3	3868.5	49.209	4	3810.65	22.9336	5	3737.37	-22.0073
6	3698.8	3.27473	7	3559.95	2.27754	8	3509.81	49.2766	9	3432.67	11.0282	10	3386.39	-29.9246
11	3324.68	-10.1367	12	3293.82	22.2803	13	3251.4	-38.1135	14	3193.54	7.94406	15	3158.83	22.0543
16	3097.12	33.7244	17	3054.69	58.1894	18	3012.27	46.6009	19	2950.55	-9.15704	20	2911.99	18.3167
21	2857.99	-7.16489	22	2653.57	62.1197	23	2514.72	53.0907	24	2449.15	66.0381	25	2314.16	84.531
26	2275.59	81.7383	27	2171.45	77.2503	28	2055.75	81.7394	29	1727.91	59.9898	30	1662.34	55.4445
31	1623.77	9.25977	32	1515.78	73.8498	33	1477.21	54.1534	34	1376.93	27.3888	35	1241.93	63.3932
36	1145.51	11.0256	37	1103.08	18.8299	38	1064.51	39.4364	39	937.235	65.0547	40	860.096	75.1505
41	817.67	64.1765	42	771.387	51.7585	43	671.106	72.6076	44	605.539	62.2209	45	509.115	85.152
46	466.689	57.284												

PHYSICAL CHARACTERIZATION

Optical Microscopy

To establish the uniformity in size distribution of the prepared microspheres the particle size distribution analysis was performed as described in the methodology by using an optical microscopy technique. The various mean diameters described were also calculated. The results revealed that the size distribution of the prepared microspheres were found to be uniform and narrow. The above result were also evident from the following normal distribution graph obtained while plotting the mean size range and number of particles. The graphs obtained were given in the following **Figure.No: 11-16**.

Fig.No. 11: Normal Frequency Distribution curve (F1-F5)

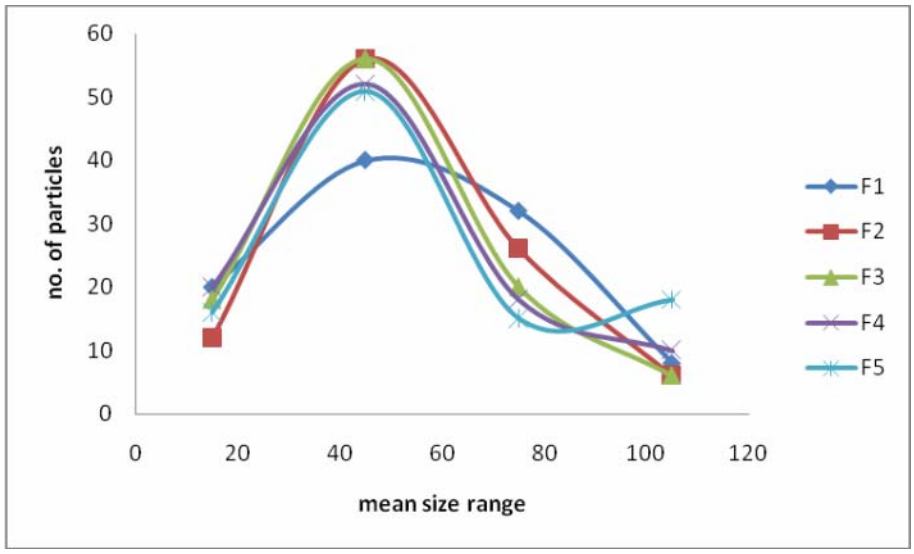


Fig.No. 12: Normal Frequency Distribution Curve (F6-F10)

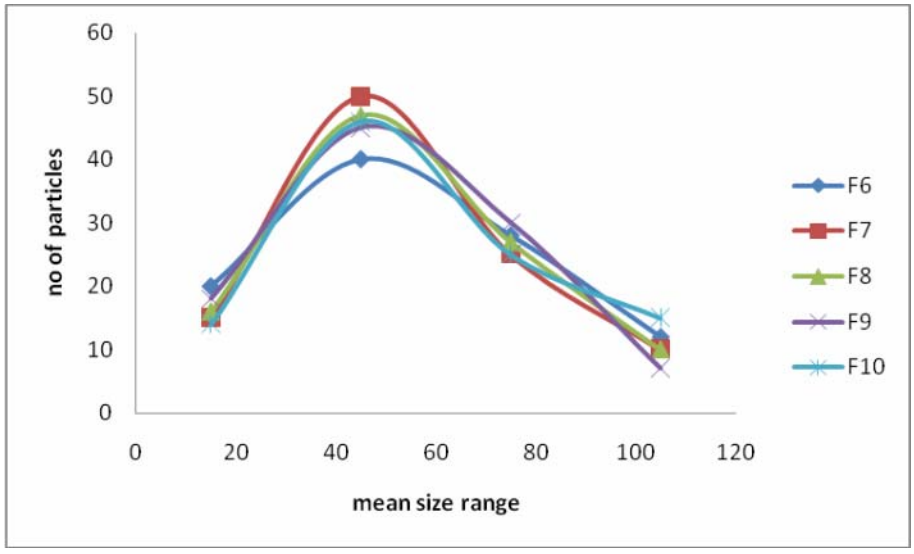


Fig.No. 13: Normal Frequency Distribution Curve (F11-F15)

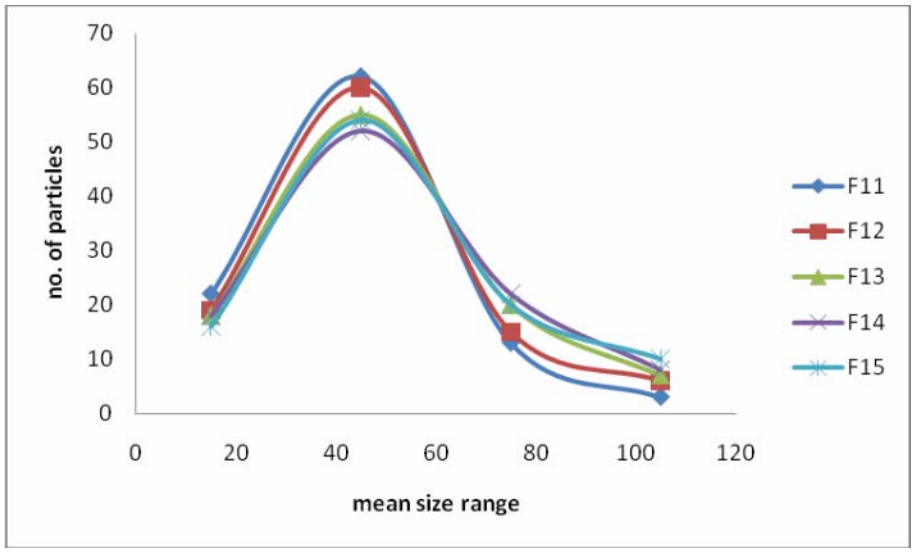


Fig.No. 14: Normal Frequency Distribution Curve (F16-F20)

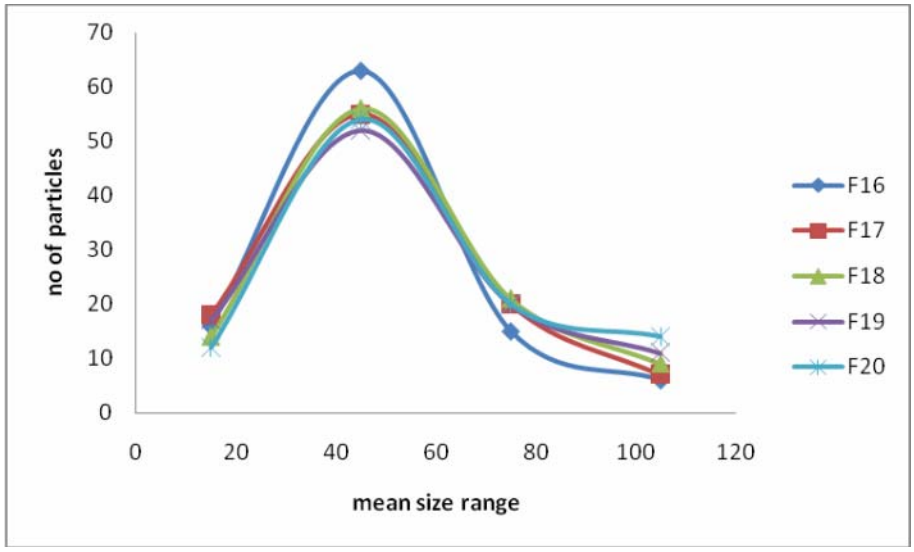


Fig.No. 15: Normal Frequency Distribution Curve (F21-F25)

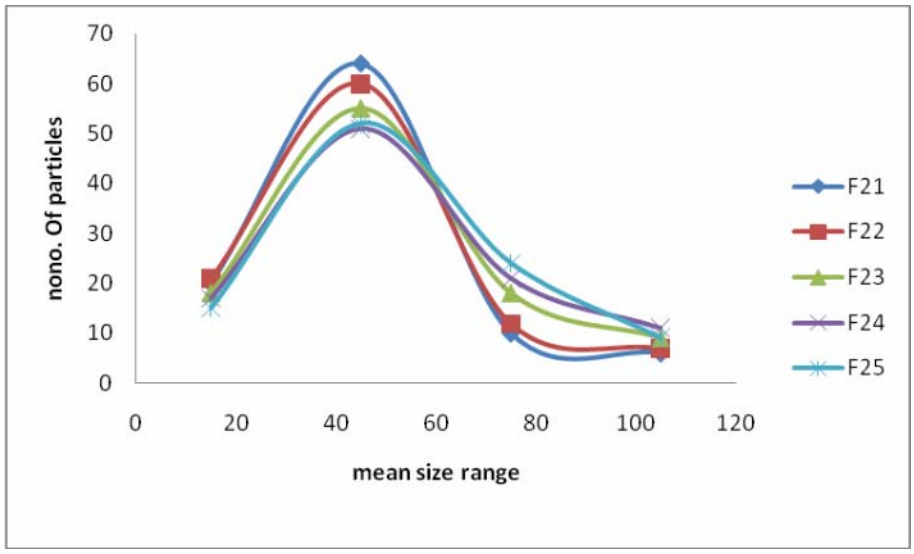
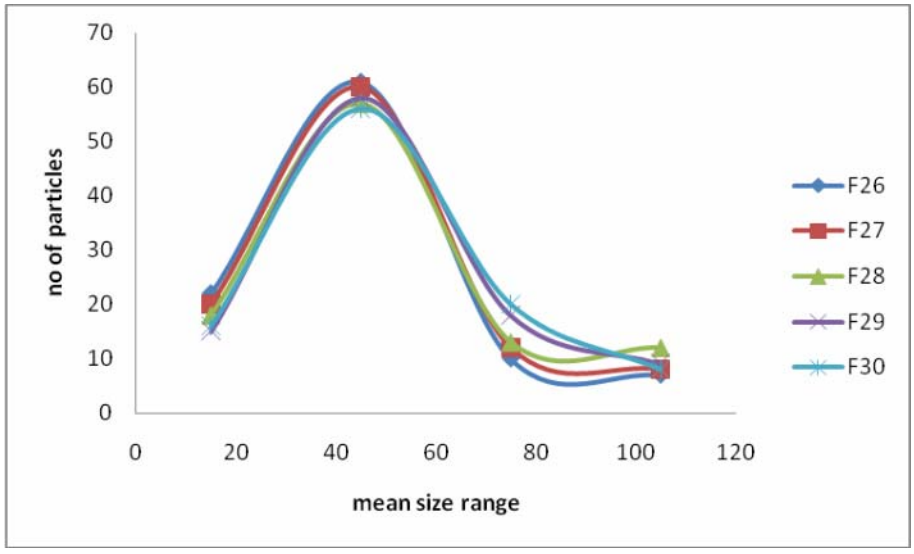


Fig.No. 16: Normal Frequency Distribution Curve (F26-F30)



EFFECT OF CONCENTRATION OF POLYMER

The effect of concentration of polymer on the size of microspheres formed were studied and it was found that there was an increase in average diameter of particles as an increase in the concentration of polymer since at higher concentration the polymer solution dispersed into larger droplets which can be confirmed from the following **table no:7**. Similar result was observed from S.Tamizharasi et al[12] where the particle size gradually increased with increasing in proportion of PCL.

EFFECT OF STIRRING SPEED

The effect of stirring speed on the size of microspheres were studied and found that there was a decrease in average diameter of the particles as the stirring speed increased which was evident from the **Table.No: 8** given below. In a similar study S.Ravi et al[13] reported that size of the microspheres was determined by the stirring speed. Stirring speed was parameter of primary importance in the emulsification step because it provides energy to disperse the oil phase in aqueous. The mean particle size of the microspheres was inversely proportional to stirring speed; consequently increase in stirring speed decreased the size of the microspheres because secondary emulsion was broken up into

smaller droplets at higher input power. But in our study the preparation of secondary emusion was not done.

Table.No. 7: Average mean diameter of the microspheres (F1-F30)

Conc of emulsifier (%w/v)	Stirring speed (rpm)	Drug to polymer ratio	Code	Length no (µm)	Surface no (µm)	Volume no (µm)	Volume surface (µm)
1.3	500	1:1	F1	49.2	54.415	63.145	85.030
		1:2	F2	50.4	56.683	62.124	74.622
		1:3	F3	52.8	57.31	61.32	70.19
		1:4	F4	53.4	59.47	64.21	74.84
		1:5	F5	55.5	62.426	68.302	81.764
	1000	1:1	F6	52.8	58.40	62.92	73.06
		1:2	F7	54	59.62	64.417	75.1898
		1:3	F8	54.3	60.07	64.87	75.65
		1:4	F9	54.6	61.26	66.506	78.65
		1:5	F10	57.3	63.42	68.50	79.92
	1500	1:1	F11	44.1	48.652	52.754	62.025
		1:2	F12	47.4	52.564	57.29	68.062
		1:3	F13	49.8	55.23	60.01	70.84
		1:4	F14	51	56.683	61.571	72.647
		1:5	F15	52.2	57.940	62.986	74.437
1.95	500	1:1	F16	48.3	53.075	57.558	67.692
		1:2	F17	49.8	55.236	60.012	70.841
		1:3	F18	52.5	57.784	62.512	73.160
		1:4	F19	52.5	58.558	63.798	75.728
		1:5	F20	55.8	61.555	66.649	78.135
0.65	500	1:1	F21	45.6	50.646	55.466	66.526
		1:2	F22	46.5	52.048	57.189	69.045
		1:3	F23	50.4	56.205	61.345	73.077
		1:4	F24	52.8	58.864	64.068	75.896
		1:5	F25	53.1	58.558	63.274	73.878
	1000	1:1	F26	45.6	51.176	56.415	68.557
		1:2	F27	47.4	53.075	58.342	70.495
		1:3	F28	50.7	57	62.684	75.810
		1:4	F29	51.3	56.683	61.577	72.668

		1:5	F30	51	56.365	61.141	71.941

Table No 8: Average mean diameter of the microspheres with respect to stirring speed

Concentration of emulsifier (%)	Drug to polymer ratio	Stirring speed	Code	Length no (µm)	Surface no (µm)	Volume no (µm)	Volume surface (µm)
1.3	1:1	500	F1	49.2	54.415	63.145	85.030
		1000	F6	52.8	58.40	62.92	73.06
		1500	F11	44.1	48.652	52.754	62.025
	1:2	500	F2	50.4	56.683	62.124	74.622
		1000	F7	54	59.62	64.417	75.1898
		1500	F12	47.4	52.564	57.29	68.062
	1:3	500	F3	52.8	57.31	61.32	70.19
		1000	F8	54.3	60.07	64.87	75.65
		1500	F13	49.8	55.23	60.01	70.84
	1:4	500	F4	53.4	59.47	64.21	74.84
		1000	F9	54.6	61.26	66.506	78.65
		1500	F14	51	56.683	61.571	72.647
	1:5	500	F5	55.5	62.426	68.302	81.764
		1000	F10	57.3	63.42	68.50	79.92
		1500	F15	52.2	57.940	62.986	74.437
0.65	1:1	500	F21	45.6	50.646	55.466	66.526
		1000	F26	45.6	51.176	56.415	68.557
	1:2	500	F22	46.5	52.048	57.189	69.045
		1000	F27	47.4	53.075	58.342	70.495
	1:3	500	F23	50.4	56.205	61.345	73.077
		1000	F28	50.7	57	62.684	75.810
	1:4	500	F24	52.8	58.864	64.068	75.896
		1000	F29	51.3	56.683	61.577	72.668
	1:5	500	F25	53.1	58.558	63.274	73.878
		1000	F30	51	56.365	61.141	71.941

Chapter 7 Results & Discussion

EFFECT OF CONCENTRATION OF EMULSIFIER

Effect of concentration of emulsifier on the size of microspheres were studied and it was found that an optimal concentration of emulsifier was required to produce the finest stable dispersion. Below this concentration the dispersed globules/droplets tend to fuse and produce larger globules because of insufficient lowering in interfacial tension, while above the optimal concentration no significant decrease in particle size was observed, because a high amount of emulsifying agent increases the viscosity of the dispersion medium[46]. In our study it was found that there were no much changes as the concentration of emulsifier increases. The average mean diameter of the microspheres range between 70µm-80µm. The same were given in the following

Table.No:9

Table No 9: Average mean diameter of the microspheres with respect to concentration of emulsifier

Stirring speed (rpm)	Drug to polymer ratio	Conc. of emulsifier (%w/v)	Code	Length no (µm)	Surface no (µm)	Volume no (µm)	Volume Surface (µm)
500	1:1	0.65	F21	45.6	50.646	55.466	66.526
		1.3	F1	49.2	54.415	63.145	85.030
		1.95	F16	48.3	53.075	57.558	67.692
	1:2	0.65	F22	46.5	52.048	57.189	69.045
		1.3	F2	50.4	56.683	62.124	74.622
		1.95	F17	49.8	55.236	60.012	70.841
	1:3	0.65	F23	50.4	56.205	61.345	73.077
		1.3	F3	52.8	57.31	61.32	70.19
		1.95	F18	52.5	57.784	62.512	73.160
	1:4	0.65	F24	52.8	58.864	64.068	75.896
		1.3	F4	53.4	59.47	64.21	74.84
		1.95	F19	52.5	58.558	63.798	75.728
	1:5	0.65	F25	53.1	58.558	63.274	73.878
		1.3	F5	55.5	62.426	68.302	81.764
		1.95	F20	55.8	61.555	66.649	78.135

SCANNING ELECTRON MICROSCOPE (SEM)

The SEM photographs obtained were given in following **Figure No: 17-20**. The photographs revealed that the surface morphology and size of the microspheres prepared as that the formed microspheres were not spherical and discrete. In a study by McGinity[47] reported that the polymer precipitation from the organic solvent phase was strongly affected by the rate of diffusion of the organic solvent into the aqueous phase. Organic solvents of low water solubility resulted in slow polymer precipitation which facilitated complete partitioning of the drug into the aqueous

phase, resulting in empty microspheres. On the other hand, highly water-miscible solvents did not form droplets but large irregular polymer agglomerates upon emulsification due to rapid solvent exchange which leads to non uniform and poor encapsulation efficiency[13]. The microspheres prepared were coalesced into a continuous mass on drying and lost their properties as individual spheres. A similar result has also been reported by Coffin[48]. The microsphere preparation was carried out without secondary emulsification step which indicates that this process is essential to break the polymer and disperse it into the fine droplets which in turn later governed by other factors like concentration of the polymer solution, molecular weight of the polymer, homogenization speed and emulsifier concentration to obtain the required particle size.

Fig.No.17: SEM Photographs of Microspheres F_1 at 500 X

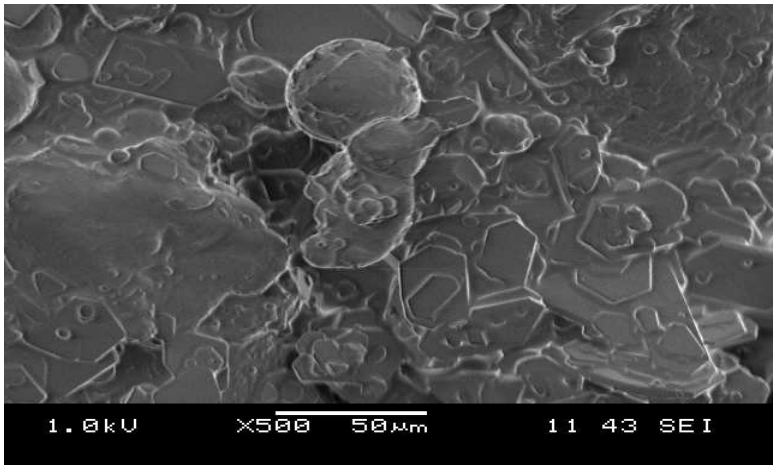


Fig.No.18: SEM Photographs of Microspheres F_5 at 300 X

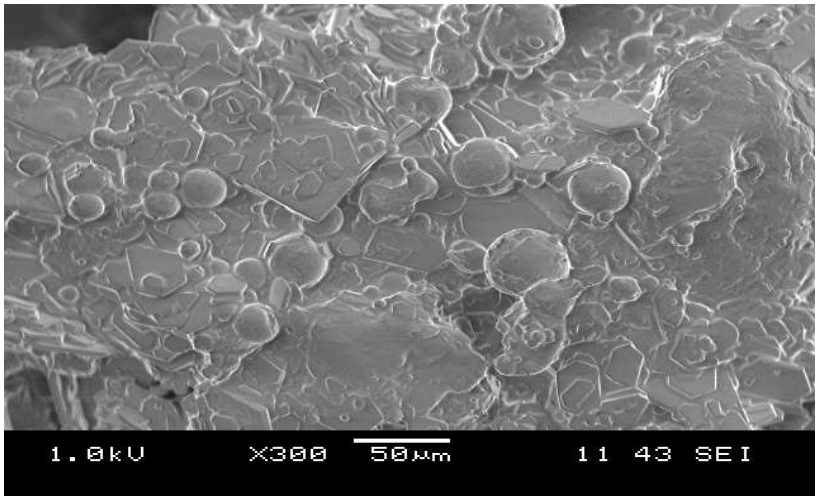


Fig.No.19: SEM Photographs of Microspheres F_{18} at 1000 X

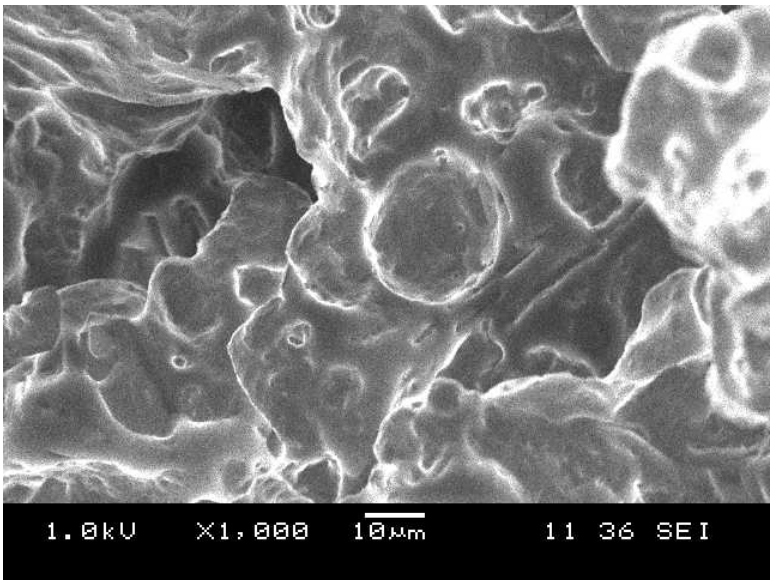
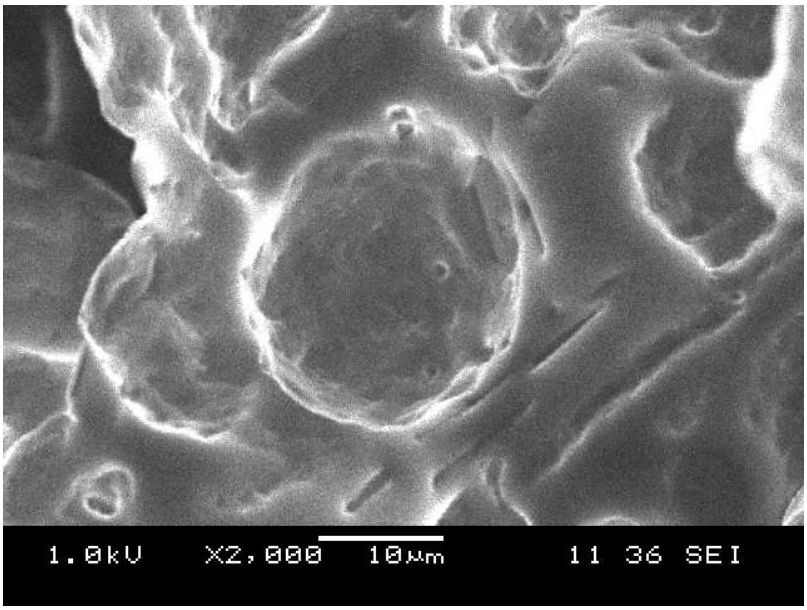


Fig.No.20: SEM Photographs of Microspheres F_{21} at 2000 X



DRUG CONTENT ANALYSIS

- **Drug actually loaded**

The amount of drug Diltiazem hydrochloride loaded to the microspheres were analysed at 236.5nm using phosphate buffer (pH 7.4) as blank. The results were given in the **Table No: 10-12**. The drug loading with respect to change in the concentration of polymer, emulsifier and stirring speed were evaluated.

EFFECT OF CONCENTRATION OF POLYMER

The average drug loading efficiency decreases with increasing concentration of polymer PCL. The encapsulation efficiency is highly influenced by the molecular weight and hydrophilicity of the polymers. This might be attributed to higher viscosity of the polymeric solution[13]. In the given **Table No:10** it was observed that when the polymer concentration was increased 5 times, there is a decrease in average drug loading efficiency that is, the drug loading efficiency decreased from 61.16% to 37.32% from F1 to F5. Similar result was observed in all set of formulations.

Table. No. 10 : Average drug loading of microspheres with respect to polymer concentration

Conc of emulsifier (% w/v)	Stirring speed (rpm)	Drug to polymer ratio	code	Average drug loading (%w/w)
1.3	500	1:1	F1	61.16
		1:2	F2	40.8
		1:3	F3	38.6
		1:4	F4	38.46
		1:5	F5	37.32
	1000	1:1	F6	36.6
		1:2	F7	35.04
		1:3	F8	30.39
		1:4	F9	27.2
		1:5	F10	25.03
	1500	1:1	F11	24.13
		1:2	F12	22.29
		1:3	F13	19.5
		1:4	F14	15.99
		1:5	F15	14.68
1.95	500	1:1	F16	22.04
		1:2	F17	20.11
		1:3	F18	10.83
		1:4	F19	5.4
		1:5	F20	2.6
0.65	500	1:1	F21	80
		1:2	F22	72.37
		1:3	F23	64.3
		1:4	F24	54.92
		1:5	F25	53.4
	1000	1:1	F26	49.46
		1:2	F27	47.8
		1:3	F28	45.37
		1:4	F29	44.15
		1:5	F30	36.8

EFFECT OF STIRRING SPEED

The effect of stirring speed on the amount of drug entrapped were also studied and found that as the stirring speed increases, the average drug loading efficiency decreases. In case of 1:1 drug to polymer ratio, it was seen that when the stirring speed was increased from 500-1500, the average drug loading efficiency decreased from 61.16% to 24.13%. Similar results were seen in all set of formulations. This is evident from the following

Table No: 11

Table.No.11 : Average drug loading efficiency with respect to stirring speed

Concentration of emulsifier(%w/v)	Drug to polymer ratio	Stirring speed (rpm)	code	Average drug loading (%w/w)
1:3	1:1	500	F1	61.16
		1000	F6	36.6
		1500	F11	24.13
	1:2	500	F2	40.8
		1000	F7	35.04
		1500	F12	22.29
	1:3	500	F3	38.6
		1000	F8	30.39
		1500	F13	19.5
	1:4	500	F4	38.46
		1000	F9	27.2
		1500	F14	15.99
	1:5	500	F5	37.32
		1000	F10	25.03
		1500	F15	14.68

EFFECT OF CONCENTRATION OF EMULSIFIER

The average drug loading efficiency of the microspheres decreases with increasing emulsifier concentration. The percent encapsulation efficiency of PCL microspheres was found to vary within 2.6 to 80%.

Table.No. 12 : Average drug loading efficiency with respect to concentration of emulsifier

Stirring speed (rpm)	Drug to polymer ratio	Conc of emulsifier (%w/v)	Code	Average drug loading (%w/w)
500	1:1	1.3	F1	61.16
		1.95	F16	22.04
		0.65	F21	80
	1:2	1.3	F2	40.8
		1.95	F17	20.11
		0.65	F22	72.37
	1:3	1.3	F3	38.6
		1.95	F18	10.83
		0.65	F23	64.3
	1:4	1.3	F4	38.46
		1.95	F19	5.4
		0.65	F24	54.92
	1:5	1.3	F5	37.32
		1.95	F20	2.6

		0.65	F25	53.4

As reported by S.Ravi et al[13] the organic solvents acetone and ethyl acetate did not encapsulate the drug efficiently and produced larger particle size of microspheres compared to dichloromethane. The only organic solvent which could successfully encapsulate higher amount of drug with smaller size of microspheres under selected experimental conditions was dichloromethane. This might be due to the optimum solubility of dichloromethane in water. These results indicated that dichloromethane is a good solvent for the formation of microspheres with high entrapment efficiency due to its desirable physical properties extremely low solubility in water, ability to dissolve large amounts of polymer and required the lowest heat of evaporation

- **DRUG RELEASE STUDIES**

The *in-vitro* drug release patterns were found by performing the experiment as described in the methodology. The results obtained were given in **Table No: 13-15** and **Figure No: 21-26**

Effect of concentration of polymer

Effect of concentration of polymer drug release were studied. The results indicated that the more sustained effect with increase

in the concentration of poly (ε-caprolactone). The *in-vitro* release profiles of microspheres are intended to assist in predicting the ultimate behaviour of the given microsphere formulation. The release of drug from the microspheres showed a biphasic profile. The microspheres showed an initial rapid release of a certain amount of drug which was deposited on the surface of the followed by a slow and continuous release which corresponds to release of drug entrapped in the microspheres. Similar result was observed by S.Ravi et al[13]. In case of F1 to F5 it was observed that the percentage of drug release decreased from 53.71 to 32.39%. All set of formulations had shown a similar report.

EFFECT OF STIRRING SPEED

Effect of stirring speed on the percentage of drug release was studied and it was found that the drug release was high as the stirring speed was increased from 500-1500.

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Table. No 13: percentage of drug release from microspheres (F1-F30)

Formulation code	Time								
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
F1	0	30.45	32.27	34.04	34.23	40.70	41.08	48.95	53.71
F2	0	27.42	29.60	31.40	32.29	36.49	41.36	46.10	47.06
F3	0	23.20	31.18	34.87	35.56	35.93	38.62	39.55	46.11
F4	0	26.36	28.43	28.56	30.03	33.38	41.29	41.40	45.23
F5	0	10.75	13.00	14.81	16.25	17.42	24.92	30.78	32.39
F6	0	36.71	37.01	37.82	38.18	42.94	44.93	47.68	54.03
F7	0	29.68	33.77	34.50	35.13	39.07	41.99	47.70	53.36
F8	0	27.25	31.99	33.92	34.49	38.24	44.16	52.01	53.21
F9	0	24.47	24.88	25.20	27.42	31.88	33.26	35.74	40.72
F10	0	21.19	21.27	28.79	29.33	29.83	32.64	33.51	34.71
F11	0	32.08	41.70	42.66	44.94	49.22	51.87	53.49	58.56
F12	0	29.04	39.67	42.20	44.04	45.72	50.27	52.18	55.51
F13	0	32.03	34.31	37.40	41.78	44.74	48.94	52.03	53.62
F14	0	20.16	24.99	25.45	29.02	31.91	34.39	35.14	37.60
F15	0	6.9	8.4	11.81	18.01	22.30	24.03	22.72	32.96

Table. No 13: percentage of drug release from microspheres (F1-F30) continues...

Formulation code	Time								
	0	1	2	3	4	5	6	7	8
F16	0	25.72	30.76	35.93	39.93	41.98	44.43	48.78	52.47
F17	0	25.18	29.47	34.01	36.76	40.92	45.65	48.44	50.22
F18	0	23.63	27.83	31.58	34.14	37.21	41.89	45.98	47.99
F19	0	19.10	22.27	24.86	26.67	27.77	32.39	36.97	40.98
F20	0	16.38	18	20.38	21.71	23.21	26.54	27.77	29.60
F21	0	21.27	22.53	23.96	24.51	25.72	39.56	50.41	61.264
F22	0	22.38	23.68	25.06	29.35	33.30	38.39	50.32	56.74
F23	0	22.79	26.89	31.32	33.25	36.98	42.49	43.21	48.97
F24	0	19.36	20.91	22.01	23.48	26.59	30.21	36.61	42.99
F25	0	18.32	18.82	20.15	22.81	25.30	27.51	30.01	36.97
F26	0	25.03	28.52	33.68	34.93	39.84	41.76	42.94	46.54
F27	0	20.11	22.38	24.89	28.79	34.28	36.11	40.80	42.98
F28	0	22.04	24.60	28.15	31.78	31.96	35.08	38.50	42.02
F29	0	22.29	25.92	28.37	29.81	33.45	35.33	37.67	38.75
F30	0	10.83	13.91	14.58	17.97	18.49	19.90	21.95	22.96

Table.No. 14: Percentage of drug release from microspheres with respect to stirring speed

Formulation code	Stirring speed (rpm)	Time (hrs)								
		0	1	2	3	4	5	6	7	8
F1	500	0	30.45	32.27	34.04	34.23	40.70	41.08	48.95	53.71
F6	1000	0	36.71	37.01	37.82	38.18	42.94	44.93	47.68	54.03
F11	1500	0	32.08	41.70	42.66	44.94	49.22	51.87	53.49	58.56
F2	500	0	27.42	29.60	31.40	32.29	36.49	41.36	46.10	47.06
F7	1000	0	29.68	33.77	34.50	35.13	39.07	41.99	47.70	53.36
F12	1500	0	29.04	39.67	42.20	44.04	45.72	50.27	52.18	55.51
F3	500	0	23.20	31.18	34.87	35.56	35.93	38.62	39.55	46.11
F8	1000	0	27.25	31.99	33.92	34.49	38.24	44.16	52.01	53.21
F13	1500	0	32.03	34.31	37.40	41.78	44.74	48.94	52.03	53.62
F4	500	0	26.36	28.43	28.56	30.03	33.38	41.29	41.40	45.23
F9	1000	0	24.47	24.88	25.20	27.42	31.88	33.26	35.74	40.72
F14	1500	0	20.16	24.99	25.45	29.02	31.91	34.39	35.14	37.60
F5	500	0	10.75	13.00	14.81	16.25	17.42	24.92	30.78	32.39
F10	1000	0	21.19	21.27	28.79	29.33	29.83	32.64	33.51	34.71
F15	1500	0	6.9	8.4	11.81	18.01	22.30	24.03	22.72	32.96

EFFECT OF CONCENTRATION OF EMULSIFIER

Formulation with low concentration of emulsifying agent also showed a fast release may be due to porous structure. It is confirmed from the given **Table No:15** that as the concentration of emulsifier increased from 0.65% to 1.95%, the release of drug from the prepared microspheres decreased from 61.26% to 52.47%.

In the present study, dissolution studies had been conducted for a period of 8 hours. During the first hour one-third of the release has taken place. A total of 50% release only had been observed during the eight hour dissolution study. Further studies could have been done by extending the time upto 16 hours for knowing the complete release pattern.

Table. No 15: percentage of drug release from microspheres with respect to concentration of emulsifier

Concentration of emulsifier (% w/v)	Formulation code	Time (hrs)								
		0	1	2	3	4	5	6	7	8
0.65	F21	0	21.27	22.53	23.96	24.51	25.72	39.56	50.41	61.264
1.3	F1	0	30.45	32.27	34.04	34.23	40.70	41.08	48.95	53.71
1.95	F16	0	25.72	30.76	35.93	39.93	41.98	44.43	48.78	52.47
0.65	F22	0	22.38	23.68	25.06	29.35	33.30	38.39	50.32	56.74
1.3	F2	0	27.42	29.60	31.40	32.29	36.49	41.36	46.10	47.06
1.95	F17	0	25.18	29.47	34.01	36.76	40.92	45.65	48.44	50.22
0.65	F23	0	22.79	26.89	31.32	33.25	36.98	42.49	43.21	48.97
1.3	F3	0	23.20	31.18	34.87	35.56	35.93	38.62	39.55	46.11
1.95	F18	0	23.63	27.83	31.58	34.14	37.21	41.89	45.98	47.99

Table. No 15: percentage of drug release from microspheres with respect to concentration of emulsifier continues..

Concentration of emulsifier (% w/v)	Formulation code	Time (hrs)								
		0	1	2	3	4	5	6	7	8
0.65	F24	0	19.36	20.91	22.01	23.48	26.59	30.21	36.61	42.99
1.3	F4	0	26.36	28.43	28.56	30.03	33.38	41.29	41.40	45.23
1.95	F19	0	19.10	22.27	24.86	26.67	27.77	32.39	36.97	40.98
0.65	F25	0	18.32	18.82	20.15	22.81	25.30	27.51	30.01	36.97
1.3	F5	0	10.75	13.00	14.81	16.25	17.42	24.92	30.78	32.39
1.95	F20	0	16.38	18	20.38	21.71	23.21	26.54	27.77	29.60

Fig.No. 21 : Drug Release of Prepared microspheres (F1-F5)

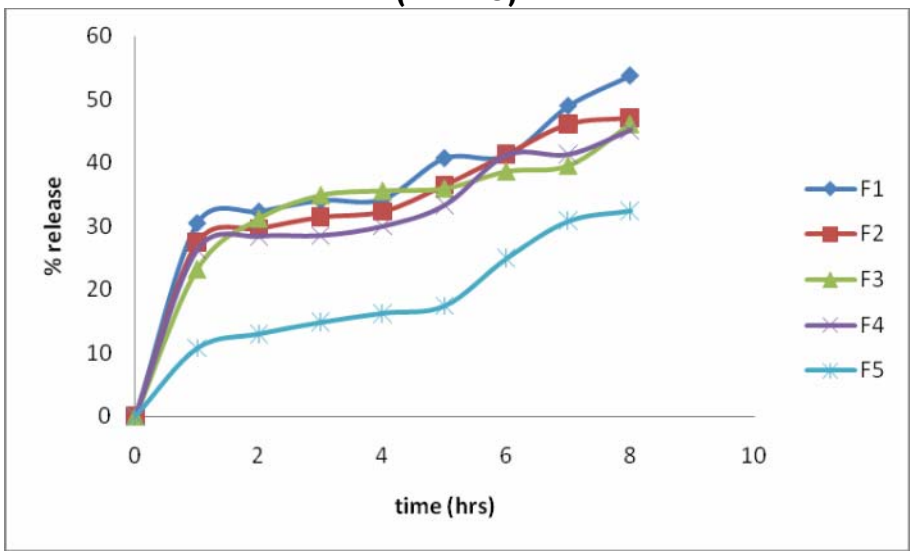


Fig.No. 22 : Drug Release of Prepared microspheres (F6-F10)

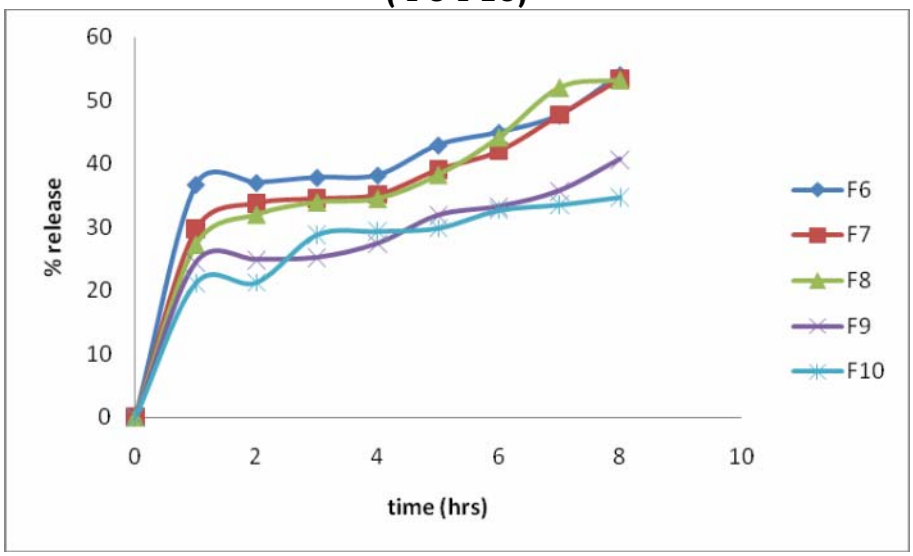


Fig.No. 23 : Drug Release of Prepared microspheres

(F11-F15)

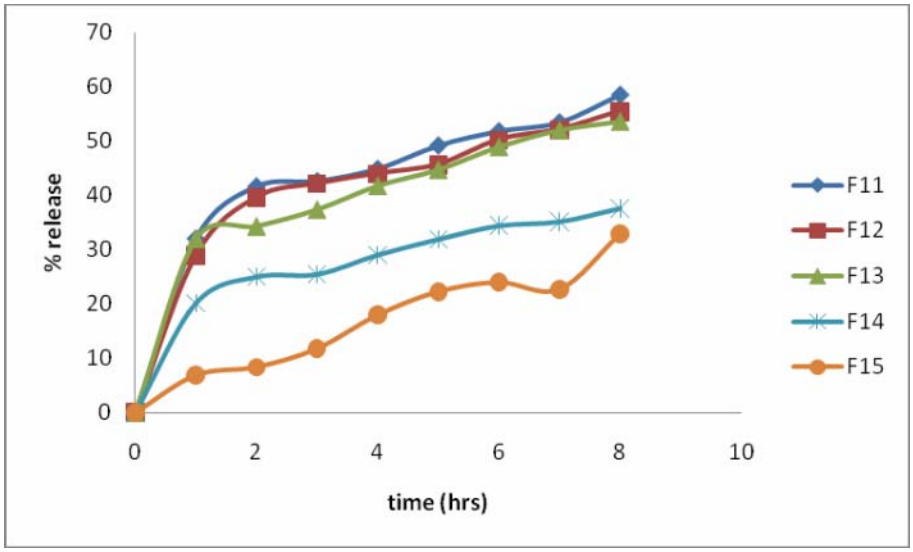


Fig.No.24 : Drug Release of Prepared microspheres
(F16-F20)

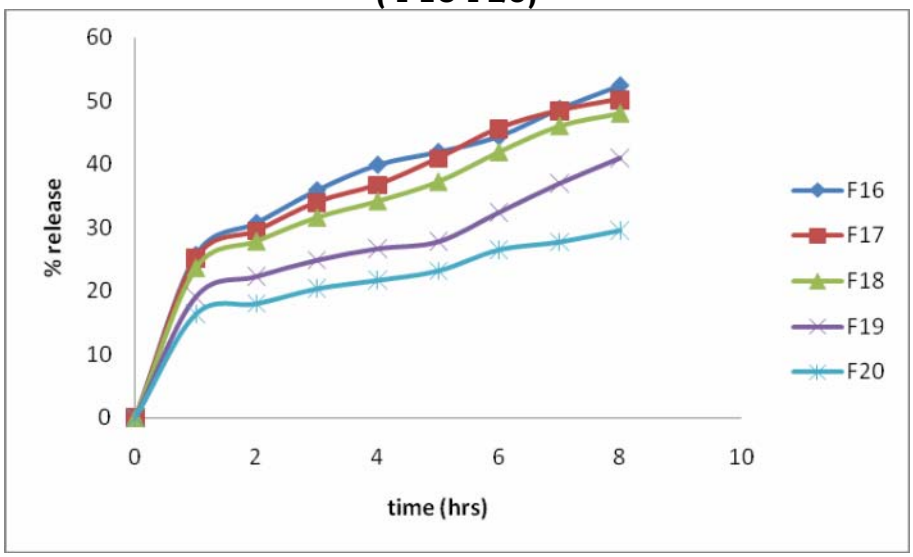


Fig.No. 25 : Drug Release of Prepared microspheres (F21-F25)

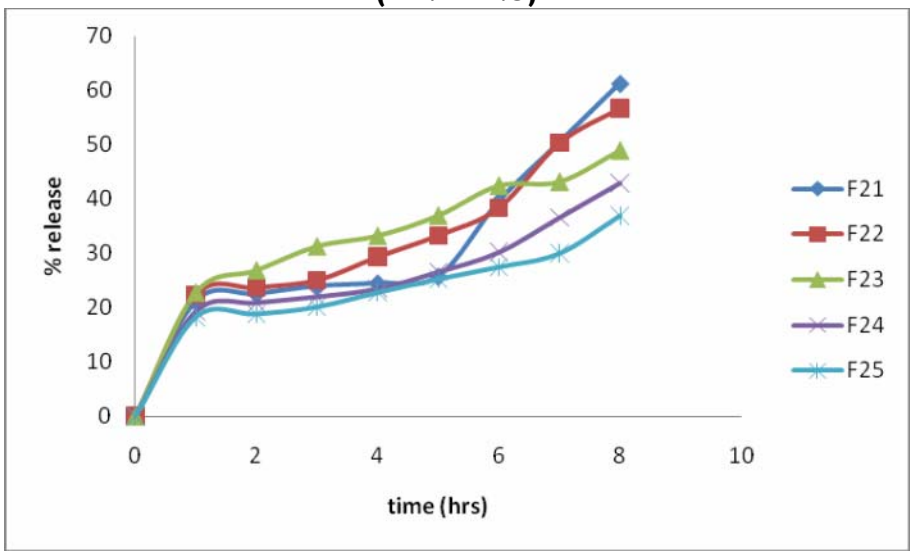
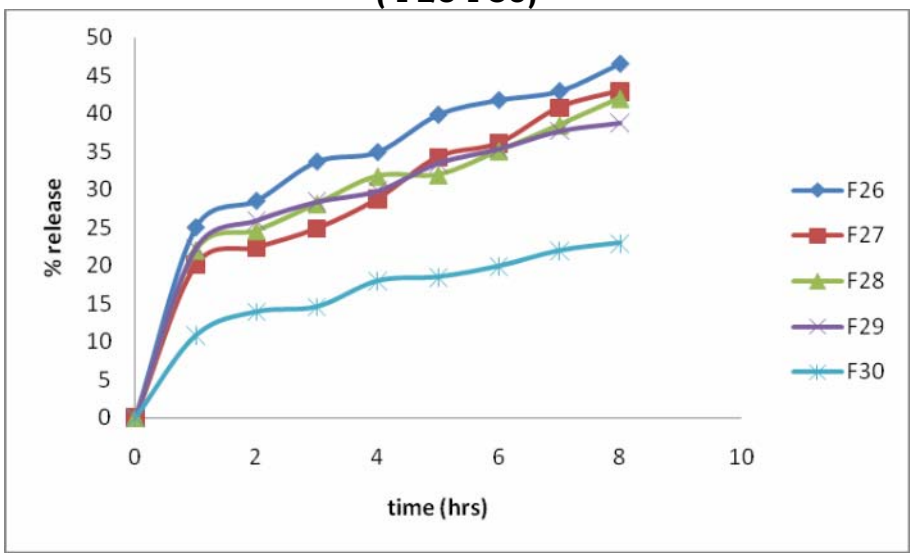


Fig.No. 26 : Drug Release of Prepared microspheres (F26-F30)



KINETICS OF DRUG RELEASE

In-vitro data obtained for Poly (ε-caprolactone) microspheres containing Diltiazem hydrochloride were used to determine the dissolution kinetics. The drug release data of Diltiazem hydrochloride were fitted to models representing Zero order (cumulative amount of drug released vs time), First order (log cumulative percentage of drug remaining vs time), Higuchi's (cumulative percentage of drug released vs square root of time), and Korsmeyer's equation (log cumulative percentage of drug released vs log time) kinetics to know the release mechanisms. The data were processed for regression analysis using MS-EXCEL statistical functions. These data indicated that the drug release followed the diffusion controlled model as described by Higuchi's square root of time equation.

Table 16: Drug release kinetics for poly (ε-caprolactone) microspheres

Formulation	Zero Order R^2	First Order R^2	Higuchi's Plot R^2	Korsmeyer's Plot R^2
F1	0.778	0.852	0.918	0.444
F2	0.784	0.856	0.933	0.451
F3	0.721	0.788	0.920	0.457
F4	0.774	0.839	0.919	0.449
F5	0.934	0.935	0.907	0.636
F6	0.65	0.741	0.849	0.394
F7	0.762	0.840	0.916	0.439
F8	0.826	0.892	0.945	0.472
F9	0.754	0.813	0.910	0.441
F10	0.713	0.765	0.918	0.457
F11	0.727	0.834	0.927	0.439
F12	0.734	0.832	0.934	0.452
F13	0.763	0.860	0.941	0.444
F14	0.778	0.837	0.954	0.477
F15	0.953	0.947	0.953	0.772
F16	0.82	0.900	0.974	0.485
F17	O.836	0.910	0.978	0.491
F18	0.844	0.911	0.976	0.494
F19	0.856	0.905	0.974	0.506
F20	0.794	0.837	0.954	0.487
F21	0.868	0.835	0.813	0.548
F22	0.910	0.909	0.908	0.541
F23	0.855	0.917	0.978	0.503
F24	0.866	0.890	0.919	0.513
F25	0.836	0.871	0.934	0.494
F26	0.783	0.857	0.958	0.470
F27	0.885	0.933	0.980	0.524
F28	0.803	0.865	0.958	0.477
F29	0.755	0.819	0.943	0.460
F30	0.835	0.865	0.978	0.550

SUMMARY

The current study "**Formulation and evaluation of Diltiazem hydrochloride microspheres for Oral Controlled Release Drug Delivery using Poly (ε-caprolactone)**" was formulated and evaluated. The results obtained from the above study can be summarized as follows.

- Literatures pertaining to microspheres, poly (ε-caprolactone) and drug Diltiazem hydrochloride were surveyed thoroughly and documented.
- Standard graph was prepared by using UV spectrophotometer with phosphate buffer pH 7.4 as solvent.
- Microspheres containing the drug and varying concentration of polymer, stirring speed and emulsifier was prepared by solvent evaporation technique.
- The prepared microspheres were evaluated.

COMPATIBILITY STUDIES

IR spectral analysis

The IR spectrums revealed that there were no interaction between the drug and polymer.

PHYSICAL CHARACTERISATION

Particle size and size distribution analysis

- ✓ The particle size distribution of each formulation was very well within the narrow size range.
- ✓ Increases in concentration of poly (ε-caprolactone) the average mean diameter of the microspheres were increased.
- ✓ When the stirring speed was increased there was a decrease in average mean diameter of the particles.
- ✓ An optimal concentration of emulsifier is required to produce the desired particle size.

Scanning electron microscope

- ✓ The formed microspheres were not spherical and discrete. The microspheres prepared were coalesced into a continuous mass on drying and lost their properties as individual spheres.

DRUG CONTENT ANALYSIS

- ✓ Increasing the concentration of poly (ε-caprolactone) resulted in decrease in drug loading efficiency.
- ✓ Increase in concentration of emulsifier, decrease in drug loading efficiency.
- ✓ As the stirring speed increases, drug loading efficiency decreases.

***IN-VITRO* DRUG RELEASE STUDIES**

- ✓ The *in-vitro* drug release studies revealed that 30-40% of drug were released in 1st hour and latter it was sustained. The release of drug from the microspheres had shown a biphasic profile.
- ✓ The microspheres showed an initial rapid release of a certain amount of drug which was deposited on the surface of the followed by a slow and continuous release which corresponds to release of drug entrapped in the microspheres.
- ✓ Increase in concentration of poly (ε-caprolactone), the drug release rate becomes more sustained.
- ✓ Drug release was high as the stirring speed was increased from 500-1500.

KINETICS OF DRUG RELEASE

- ✓ The results indicated that the drug release from formulation followed the diffusion controlled model as described by Higuchi's square root of time equation.

CONCLUSION

The work entitled **"Formulation and Evaluation of Diltiazem hydrochloride Microspheres for Oral Controlled Release Drug Delivery using Poly (ε-caprolactone**)" was formulated and evaluated. The study can be concluded that from the investigations a proper selection of formulation parameters are important to achieve the desired particle size, drug loading efficiency and to sustain the release of drug from PCL microspheres. It was observed that particle size increased as the polymer concentration increased. A secondary emulsification process is required where the particle size can be further reduced which was not carried in our present work. Increase in stirring speed also decreases the particle size. In case of drug loading efficiency, when the concentration of polymer and emulsifier was increased, the drug loading efficiency decreased. Increase in stirring speed also caused a decrease in drug loading efficiency. The release of drug from the microspheres was sustained when the concentration of polymer is increased. When the stirring speed was increased from 500-1500 rpm, the drug release was high. The *in-vitro* release profiles of drug from all formulations could be best expressed by Higuchi's equation.

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LIST OF FIGURES

LIST OF TABLES